Develop New or Improved Instruments and Technologies for Use in Research and Medicine

SCIENCE ADVANCES

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Teaching a Computer to Diagnose Airway Disease

Background: In the 1970=s, CT (computed tomography) scan technology, based on mathematical algorithms developed at the turn of the century and then state-of-the-art computers, gave physicians an unparalleled view inside the human body. Soon thereafter, physicians began to generate three-dimensional computer models of body organs, using mathematical techniques developed by computer scientists and mathematicians.

In 1989, engineers and physicists at medical equipment manufacturer research labs developed Ahelical@ CT scanners. In 1994, physicians made use of these new generation CT scanners to acquire detailed images of a large body region during a single breathhold. These physicians turned these images into elaborate three-dimensional models of the interior of anatomic structures in a way that simulated conventional endoscopy.

Advance: This year NIH researchers developed and published a method to use these simulated endoscopies to automatically locate tumors in the air passages of the lungs. Their methods are based on the abnormal shape of airway tumors, use mathematical techniques developed by 19th century mathematicians, and build on more recent image processing methods developed by mathematicians and computer graphics specialists.

Implications: The significance of these methods is that they may allow physicians to diagnose tumors of the airway without the need to pass an instrument down a patient=s throat to see the tumor directly. We are also studying whether these methods can be used to find tumors in other organs without the need for conventional endoscopy, such as colon polyps which are precursors to colon cancer. [secondary B diagnosis]

Summers RM, Selbie WS, Malley JD, Pusanik LM, Dwyer AJ, Courcoutsakis NA, Shaw DJ, Kleiner DE, Sneller MC, Langford CA, Holland SM, Shelhamer JH. Polypoid lesions of airways: early experience with computer-assisted detection by using virtual bronchoscopy and surface curvature. *Radiology* 208:331-337 (1998).

Novel Imaging Technology for Joint Disorders

Background: Optical coherence tomography (OCT) is a new method of imaging capable of detecting small structural changes that occur in tissues during the earliest stages of disease. The resolution of the OCT system is typically on the order of 5-15 microns (one micron is equal to one millionth of a meter), which is nearly tenfold greater than the resolution of any available clinical technology. OCT was originally developed to image the transparent tissue of the eye, and clinical studies are in progress evaluating its potential for a wide range of eye diseases. In addition to its high resolution, several features make OCT attractive for joint imaging: it is optical fiber based, allowing easy integration with an arthroscope, an instrument for the examination of the interior of a joint; it is compact and portable, making it well suited for an outpatient setting; it is Anoncontact,@ allowing imaging to be performed through air or a transparent medium such as saline; it can be performed at high speeds, allowing information at the cellular level to be obtained from throughout the joint; and it can be used in combination with other techniques, providing biochemical and structural information from tissue.

Advance: Using normal and osteoarthritic cartilage specimens, investigators demonstrated the usefulness of this technology to detect structural abnormalities in cartilage. There was a strong correlation between tissue structure observed in OCT images and the corresponding microscopic examination of the tissue. This included identification of irregularities in cartilage surface and identification of new bone growth. These changes occur early in the pathogenesis of osteoarthritis and before loss of cartilage thickness (narrowing of the joint space, as seen by radiography).

Implications: OCT represents a promising new technology for detecting cartilage abnormalities through ultrahigh-resolution joint imaging. Refinement of this technology and its ultimate clinical use may permit early diagnosis of degenerative joint disorders, evaluation of disease severity and progression, and enhanced understanding of pathological processes within joints.

Herrmann JM, Pitris C, Bourma BE, Boppart SA, Jesser CA, Stamper D, Fujimoto JG, and Brezinski ME: High-resolution imaging of normal and osteoarthritic cartilage with optical coherence tomography. J Rheumatol, in press.

A New View of the Eye

Background: Researchers at the University of Rochester have shown that it is possible to image the three photoreceptor cone types in the intact living retina with a technology called adaptive optics. Adaptive optics were originally developed for astronomy: telescopes equipped with adaptive optics permitted the capture of clear images despite a turbulent atmosphere. Recently, this technology has been applied to the visual system.

Advance: Adaptive optics technology allows us to see clearly into the human eye, giving the clearest views yet of the living retina inside the eye. Here=s how it works. A high-resolution fundus camera is equipped with adaptive optics. If the cornea and lens of the eye were perfect, light from a single point on the retina would form a perfect set of plane waves as they left the eye. However in real eyes, the waves leaving the eye are distorted. The key to adaptive optics is to measure the shape of these waves with a device called a wave front sensor and then to flatten the waves back into a plane wave. This instrument flattens the waves with a deformable mirror. This mirror is an aluminized glass mirror with 37 pistons mounted on its back surface. These pistons push and pull to shape the mirror in just the right way to compensate for the eye=s aberrations.

Implications: The clinical and research applications of adaptive optics are being explored. Individuals who view the world through adaptive optics will have much sharper vision, even if they don=t need glasses. When looking into the eye, adaptive optics allow one to have a much sharper image of the retina than previously possible, to the point of resolving single cells. One can apply a technique called retinal densitometry in combination with adaptive optics to identify a photopigment type unique for each cone. Since this technology is noninvasive, a particularly exciting feature of this approach is that perceptual measurements can be made in the same eyes for which the retinal physical mosaic is completely specified. For example it is now possible to examine the integrity of photoreceptors and other cell types in the living eye, to track the progression of a number of retinal diseases such as retinitis pigmentosa, or evaluate the efficacy of rescue of cell types in the retina. Also, just as adaptive optics can be used to remove the aberrations for light leaving the eye when viewing the retina, it can also be used to remove aberrations for light entering the eye resulting in optics of unprecedented clarity.

Roorda A, Williams DR: The arrangement of the three cone classes in the living human eye. Nature 397:520-22, 1999.

Early Identification of Hearing Impairment

Background: NIH-supported research shows that detection of hearing impairment and intervention within the first six months of life leads to development of better language skills. In March of 1998, the NIH convened a Working Group on Early Identification of Hearing Impairment to provide advice on the most pressing research questions regarding diagnostic and intervention strategies following neonatal hearing screening. Based on these research recommendations, a Program Announcement with Set-Aside Funds was published in October 1998 for FY2000 funding for grant applications focusing on intervention strategies following identification of neonatal hearing impairment. Applications were encouraged that address relevant issues including, but not limited to: hardware (hearing aids, cochlear implants and other sensory aids); behavioral treatment programs; development of outcome measures to determine the benefit of intervention strategies; and, studies on the efficacy of intervention. Approximately $1 million in direct costs has been made available for the first year of support. It is anticipated that up to five awards will be made. Applications in response to the first of three receipt dates, (Feb 18, 1999, Jun 18, 1999, Oct 18, 1999) have now been received and reviewed. We anticipate funding one application from the first submission round and expect to receive additional meritorious applications in the near future.

Advance: Data collection was completed in 1998 for a five-year multi-center study on neonatal hearing impairment. The goal of this study is to develop optimal procedures for neonatal hearing screening. This study is the first truly randomized controlled comparison of three different measures of physiological response to hearing in neonates, validated by an independent measure of hearing. This is in sharp contrast to previous studies in which only those infants who Afailed@ the neonatal hearing screening test were followed. All three measures performed equally well in a variety of test environments, including the NICU, well-baby nursery and outpatient clinics, at predicting hearing status at 8-12 months of age.

Implications: The data demonstrate that a significant group of infants whose hearing is normal at birth appear to lose hearing during the first year of life. It will be necessary to monitor auditory function during this period to ensure that hearing loss is detected in a timely fashion.

Yoshinaga-Itano C et al: Language of early- and later-identified children with hearing loss. Pediatrics 102: 1161-1171, 1998.

The Hearing Aid Clinical Trial:
A multi-center, double-masked study of hearing aid benefits

Background: Hearing aids are the main form of help for persons with hearing loss. Although numerous studies have suggested that hearing aids provide significant benefit, carefully controlled, large-scale clinical trials have not been conducted. A multicenter clinical trial was conducted to compare the efficacy of three commonly used hearing aid circuits: peak clipping, compression limiting, and wide-dynamic range compression.

Advance: Patients (n = 360) with bilateral, sensorineural hearing loss were studied using a double masked, three-period, three-treatment crossover clinical trial design. The patients were fit with each of three programmable hearing aid circuits. Outcome tests were administered in the unaided condition at baseline and then at three-month intervals, in both aided and unaided conditions. The outcome test battery included tests of speech recognition, sound quality and subjective scales of hearing aid benefit. Each hearing aid circuit improved speech recognition, with improvement observed for soft and conversationally loud speech in both quiet and noisy listening conditions. In addition, a significant reduction in the problems encountered in communication was observed. Differences were found when the three circuits were compared, but most were small.

Implications: The most important result of this clinical trial is the clear and unequivocal demonstration that hearing aids provide significant patient benefit both in quiet and in noise. Most statistically significant differences between circuit types on measures of speech recognition and subjective ratings of benefit were small and lacking in clinical importance. Analyses of subgroup data, however, may yield important clinical insights for the evaluation and fitting of hearing aids.

Larson VD et al: The NIDCD/VA hearing aid clinical trial: A multi-center, doubled-masked study of hearing aid benefit. Submitted to NEJM, August 1999.

Cochlear Implants

Background: The cochlear implant is the only neural prosthesis in widespread clinical use, with over 20,000 users. This device converts sound into a pattern of electrical impulses that stimulate the auditory nerve directly, thereby bypassing the dysfunctional hair cells and restoring perception of sound. Approximately one half of the current cochlear implants recipients are children. The decision to try a cochlear implant implies a desire on behalf of the parents to have the child fully participate in the hearing world, with spoken language skills. Thus, one of the expected benefits of cochlear implantation in children is the acquisition of spoken language. A study examining language development in children with cochlear implants has recently been concluded.

Advance: This study measured language achievement in children with cochlear implants and used two comparison groups. One group consisted of students who were enrolled at a state school for the deaf and the second group were children using hearing aids who were similar to the children with cochlear implants. Both comparisons showed significant differences in English language achievement levels favoring the children using cochlear implants. Thus, improvements in speech perception and speech production seen in children using cochlear implants are being translated into improved language performance. Most gains occurred in the first two years after implantation, suggesting that children who are failing to benefit from the implant can be identified soon after implantation, allowing additional efforts to be directed toward their language development. These researchers noted that there were substantial differences in benefit appreciated by different cochlear implant users. Research to determine the causes of wide variation in performance among individual cochlear implant users is a high priority. This information is crucial for determination of implant candidacy as well as for predicting benefit and efficacy for those individuals considering cochlear implantation for either themselves or their children.

Implications: Investigations studying patients with cochlear implants in both ears and the potential for better speech perception in noise are in progress. One investigator has recently implanted five subjects in both ears. All subjects display improved ability to localize sounds and have a definite advantage in noisy environments. Preliminary data show that these individuals are able to integrate information from the two independent cochlear implants, indicating the potential for improved performance. [secondary B treatment]

Tomblin JB et al: A comparison of language achievement in children with cochlear implants and children using hearing aids. Journal of Speech, Language and Hearing Research 42: 497-511, April 1999.

Bioengineering

Background: An NIH Small Business Innovative Research (SBIR) grant program is used to help small businesses develop devices that help individuals with communication disorders. Several SBIR projects have resulted in commercial products. Two representative examples are discussed below.

Advances: ›The Montgomery Thyroplasty Implant System=, is the first standardized thyroplasty implant system. The device is used for the treatment of vocal fold paralysis and has several advantages over more traditional clinical approaches. These advantages include:

- $ a series of male and female standardized implants, eliminating the need to hand-fashion silicone block implants at the time of the surgery (reducing both trauma and surgery time);
- $ an easily inserted implant that snaps into place without sutures;
- $ a design that closes the posterior commissure giving better postsurgical results; and,
- $ if needed, easy device removal or replacement without damage to either cartilaginous or soft tissue structures.

›FlightSound= is a cost-effective and user-friendly listening system, specifically designed to interface with existing airplane sound systems. This system allows individuals with hearing impairment to receive pilot and flight attendant announcements, other audio inputs available on aircraft, and to communicate with neighboring passengers. FlightSound integrates a noise-canceling microphone design, audio amplification, signal processing and infrared transmission methodology packaged into a portable battery-powered assistive listening device. The project brought together a unique alliance between two assistive listening device companies, a major airline, federal agencies, and consumer advocate groups. Given that individuals with normal hearing also find the acoustic environment of airplanes difficult for communication, this technology might well benefit many airline passengers.

Implications: Technical innovation and federal legislation have resulted in the development of a wide range of assistive technologies that are improving the quality of life for many people. Better auditory communication on airplanes is now a reality, offering the potential to ease communication stress and enhance the comfort and security of millions of travelers with hearing problems. [secondary B treatment]

Lederman N: Flying the accessible skies! Testing a new assistive listening device for air travel, The Hearing Review, p.33, November 1998.

Breakthroughs in Assisted Reproductive Technology Have Many Applications

Background: Assisted reproductive technology (ART) is one of the miracles of modern science, based on advances in cellular and molecular biology. One of the newest ART methods to emerge is Aintracytoplasmic sperm injection@ (ICSI), in which an egg is fertilized in the laboratory, through direct injection of a sperm, and then transferred to the mother. ICSI is used to overcome male infertility, which may be linked to anomalies on the Y chromosome that cause low sperm counts and sperm immobility, and are also associated with other disorders. For instance, men who have a congenital absence of the *vas deferens*Bthe ducts in which seminal fluid is carriedBappear more likely to carry the gene for cystic fibrosis. In this example, using ICSI would perpetuate the cystic fibrosis gene. Researchers also believe that the ICSI procedure itself may cause chromosomal anomalies in offspring. Unfortunately, the use of ICSI in humans has increased in the absence of extensive animal studies and, at this time, no good animal model exists to advance this research. In a related but different challenge, difficulties involving reproductive technologies also make it hard to create transgenic animal models other than mice, even though other species, such as monkeys, may provide a better model of human disease.

Advance: For the first time, researchers have successfully refined ICSI procedures to produce rhesus monkeys. In the process, the researchers identified critical problems that may have relevance for human infertility treatments. For instance, factors were identified that could pose problems in properly positioning the injected sperm in the egg, with such misplacement jeopardizing further embryonic development. ICSI was also confirmed to cause abnormal events involving the sperm that could eventually lead to chromosomal anomalies.

In separate studies, researchers overcame difficulties in creating transgenic animals in other mammalian species by modifying and improving ICSI procedures that introduce foreign genes into eggs. By binding DNA to sperm heads alone, and injecting them directly into unfertilized mouse eggs, transgenic mice were produced with the specific genes in question in all their cells, including reproductive ones. As a result the genes could be transmitted to future generations.

Implications: The live birth of the first monkeys using ICSI successfully extends this method to non-human primates. These monkeys will provide the first closely-linked models to study the critical implications of using ICSI to overcome male infertility in humans, and will also enable rare and endangered species, as well as unique animals, to be preserved. In the process, suggestions of possible human complications associated with ICSI have been identified or confirmed, laying the foundation for future studies. Complementing these achievements, the refined ICSI procedure promises to produce better models of human disease, especially in species more closely related to humans.

Hewitson L, Dominko T, Takahashi D, Marinovich C, Ramalho-Santos J, Sutovsky P, Fanton J, Jacob D, Monteith D, Neuringer M, Battaglia D, Simerly C, and Schatten G: Unique checkpoints during the first cell cycle of fertilization after intracytoplasmic sperm injections in Rhesus monkeys. Nature Medicine 5: 431-433, 1999.

Perry ACF, Wakayama T, Kishikawa H, Kasai T, Okabe M, Toyoda Y, and Yanagimachi R: Mammalian transgenesis by intracytoplasmic sperm injection. Science 284: 1180-1183, 1999.

Functional Arteries Grown In Vitro

Background: Small-diameter blood vessel grafts are critical for the treatment of peripheral vascular and coronary artery disease. Grafts currently in use for bypass surgery are either obtained from the patient=s own vessels or constructed of synthetic materials. Although widely used and generally effective there are problems with vessel availability and high rates of failure due to formation of clots and other blockages. To overcome these obstacles, researchers have turned to tissue engineering methods to grow blood vessel grafts that have an improved capacity to remain open to blood flow.

Advance: Using a novel Abioreactor@ system that mimics the fetal cardiovascular environment, scientists took cells from adult pig arteries and grew them in a biodegradable framework under pulsatile conditions imitating that of a heart beat. After 8 weeks of culture, the appearance and behavior of the vessels were similar to those of native arteries. The vessels were then implanted into pigs where they appeared to function like normal arteries for up to 24 days. Future studies are required to assess the biological function of these vessels during long-term implantation, but the feasibility of culturing implantable arteries grown from adult cells has been demonstrated and the potential for eventually bringing this into the clinical arena is exciting.

Implications: Atherosclerosis is the major cause of mortality in the United States, and adequate grafts to serve as bypass conduits to treat this disease are lacking. The goal of this work is to provide a viable treatment option to patients with diseased heart or leg arteries by producing arteries grown in vitro using novel tissue engineering techniques. If future studies demonstrate the reliability of this method, surgeons may be able to use blood vessels grown from the patient=s own cells to replace clogged arteries. From a broader perspective of tissue engineering, the feasibility of producing an organ based on technologies that emulate natural processes has been established, making it more realistic to expect that similar techniques could lead to the production of whole organs such as hearts, livers, and kidneys. [secondary B treatment]

Niklason, LE, et al.: Functional arteries grown in vitro. Science 284: 489-97, 1999.

Ferber, D: Tissue engineering: Lab-grown organs begin to take shape. Science 284: 422-26, 1999.

New Strategy Shows Potential for Drug Delivery and Gene Therapy

Background: Drug delivery strategies have two major goals: targeting drugs to specific cells in the body and effectively delivering drugs into the cells. Research has focused on using antibodies to target drugs to specific sites in the body. To target drugs to blood vessels, for instance, investigators have used antibodies that recognize and bind to proteins on the surface of endothelial cells (the cells that line blood vessels), but these antibodies are not always efficiently internalized by the cells. Improving the cellular uptake of these antibodies has been the goal of considerable research.

Advance: Investigators recently devised a new strategy whereby an antibody that is inefficient as a drug carrier is converted into an efficient carrier for drug-targeting strategies in the lung. They used an antibody that binds strongly to a specific protein that is expressed at high levels on the surface of endothelial cells in the lung. But despite strong binding, the antibody does not become internalized in the cells efficiently and does not accumulate in the lung. By linking this antibody to a common pair of chemical compounds, investigators increased the cellular uptake of the antibody. They then tested the functional significance of these antibody carriers by linking an enzyme that inhibits oxidation to the antibody carrier and assessing its activity within the cells. Results showed the enzymeBantibody complex was internalized in endothelial cells in culture, and the cells were protected from chemically induced oxidative injury. Further, it was demonstrated that the antibody carriers accumulated in rat lungs following intravenous injection, and the lungs were protected from induced oxidative vascular injury.

Implications: Results from this research have enormous potential for improving drug delivery and gene therapy. First, this technique has direct treatment potential in disease conditions associated with oxidative endothelial injury, such as acute respiratory distress syndrome. Second, since other enzymes have been shown to be active when linked to the antibody complex, the delivery of many different therapeutic and experimental agents seems likely. In addition, experiments have demonstrated that this technique may be applicable to a variety of target cells, including tumor cells or HIV-infected cells. Also, this technique has potential for vascular gene therapy since preliminary data show that DNA linked to the antibody complex can be efficiently internalized by cells.

Muzykanov VR, Christofidou-Solomidos M, Balyasnikov I, Harshaw DW, Schultz L, Fisher AB, Albelda SM: Streptavidin facilitates internalization and pulmonary targeting of an anti-endothelial cell antibody (platelet-endothelial cell adhesion molecule 1): a strategy for vascular immunotargeting of drugs. Proc Natl Acad Sci USA 96:2379-2384, 1999.

Stem Cell Enhancement Offers New Avenue for Gene Therapy

Background: Researchers are working to overcome two major impediments to the development of gene therapy for diseases associated with cells of the blood. The first is the low efficiency of gene transfer into blood cells that is currently achievable with retroviral gene therapy vectors (transfer vehicles) in large animal models and man. The second is poor production of the protein encoded by the transferred gene. Attempts to address the first impediment have generally focused on developing new vectors or gene transfer protocols, but one investigator has recently come up with an alternative approach.

Advance: The investigator has developed a successful strategy for increasing the abundance of gene-modified hematopoietic (blood-forming) cells in experimental animals. He transferred into bone marrow stem cells a gene that confers resistance to a particular drug and then transplanted the cells into mice. When the mice were treated with the drug, unmodified stem cells were killed and, therefore, the concentration of gene-modified, drug-resistant stem cells increased. Using this approach, he demonstrated the feasibility of enriching gene-modified cells up to 10-fold, ultimately achieving up to 50 percent vector-positive cells in the peripheral blood. This procedure was shown to be safe and relatively non-toxic, and the increased abundance of gene-modified cells was apparent for the lifetime of the animals.

Implications: This research advance may make possible therapeutically useful levelsBlevels not previously achievableBof gene-corrected blood cells and thereby greatly increase the chance of success of genetic approaches to treat blood diseases such as sickle cell anemia, Cooley-s anemia, Fanconi anemia, chronic granulomatous disease, and some types of severe combined immunodeficiencies. [secondary B treatment]

Allay, JA et al: In vivo selection of retrovirally transduced hematopoietic stem cells. Nature Medicine 4:1136-1143, 1999.

A New Screening Tool for Lung Cancer

Background: Lung cancer, the leading cause of cancer death for men and women in the United States, claims the lives of an estimated 160,000 people in this country annually. This troubling statistic stems in large measure from our limited ability to detect lung cancer at an earlier and potentially more curable stage. Using available detection methods, most people are diagnosed in advanced stages of disease and only slightly more than 12 percent survive 5 years. Survival improves dramaticallyBto 70 percentBwhen the disease is identified and treated early. Clearly, an effective screening tool for lung cancer would enable early detection and reduce the number of lung cancer deaths, but until recently, none has been available to physicians. Annual chest x-rays, for example, have not been shown to be useful and alternative methods are too costly to be used for routine screening. Now, advances in imaging technology have led to the development of a promising technique, low radiation dose spiral computed tomography (spiral CT). Spiral CT can scan the entire lungs, from the neck to the diaphragm, in less than 20 secondsBa single breath-hold. Rapid scanning minimizes radiation exposure and improves the detection of smaller lesions since they are not moving in and out of the field of view due to breathing. This new tool may prove to be the first screening method to find some types of lung tumors early and reduce lung cancer deaths.

Advance: In the Early Lung Cancer Action Project study, NIH-supported researchers recently tested the effectiveness of spiral CT as a screening tool for lung cancer. The 1000 symptom-free participants of the study, considered to be at high-risk for lung cancer because of their age (60 years or older) and cigarette smoking history (a minimum of 10 pack-years; one pack year is equivalent to one pack of cigarettes smoked per day for one year), were given baseline and annual spiral CT examinations. The researchers demonstrated that, compared to chest x-ray, spiral CT is a considerably more effective tool for detecting small non-calcified lung nodules and thus, for detecting lung cancer at an earlier and potentially more curable stage. Malignant tumors were detected four times as often with spiral CT as with chest x-ray, and stage 1 tumors were detected six times as often with spiral CT as with chest x-ray. This technology, however, may be less useful in clinical application for detecting central airway tumors, such as squamous cell carcinomas, and very rapidly growing carcinomas, such as small cell lung cancers.

Implications: The opportunity to use spiral CT to safely and reliably screen for lung cancer offers new hope to those who are at increased risk for this disease. The cost of this technique is only slightly more than conventional chest x-ray. In addition, elective surgery of small stage 1 lung cancers is less costly than treating later-stage lung cancers. Further research is needed to determine whether lung cancers detected through routine screening actually are more effectively treated and how the size of the tumor at the time of detection may affect the rate of cure. Yet, by enabling physicians to find lung cancer at an earlier and more curable stage, routine screening with spiral CT promises to reduce the number of lives lost to lung cancer. [secondary B diagnosis]

Henschke CI, McCauley DI, Yankelevitz DF, et al.: Early Lung Cancer Action Project: Overall design and findings from baseline screening. Lancet 354: 99-105, 1999.

Strategies for Mapping Common Human Disease Genes

Background: Finding the genes that cause susceptibility to mental illnesses has proven extremely challenging. These common diseases have a complex basis that includes both complicated genetic components and environmental causes, both of which contribute to an individual's likelihood of developing a mental disorder. So far, traditional genetic approaches, successful at identifying the underlying cause of many rare single-gene disorders, have failed to find susceptibility genes for mental disorders.

Recently researchers have focused on whole-genome association studies, which look for differences in the frequency of genetic variants between unrelated individuals with specific illnesses and normal controls, to map the genes for common diseases. Such studies employ a genetic map of SNPs (single nucleotide polymorphisms) to detect association between a genetic marker and a disease. A SNP is a place in the genetic code where DNA differs from one person to the next by a single letter, resulting in slight genetic variations between human beings that may predispose some people to disease. In conjunction with new DNA microchip technology, SNPs will allow high-through-put screening of the entire genome of a large number of people affected by common diseases. This systematic and thorough approach has huge potential for locating the susceptibility gene for diseases of complex origin. However, estimating the number of SNPs necessary to find susceptibility genes is an essential issue.

Advance: This NIH-supported study uses computer simulations of human population history to predict how many SNPs would be needed to find genes for common diseases. The investigator estimated the numbers of SNPs required for an outbred population like the United States, as well as the numbers of SNPs needed for detecting association between a SNP and a disease in different theoretical inbred populations. In both cases, the study demonstrated that a SNP is needed about every 6,000 base pairs to find significant association with a disease gene. Given that the human genome contains 3 billion base pairs, half a million SNPs will be required for whole-genome association studies in both outbred and inbred populations.

Implications: Systematic whole-genome association studies of common diseases will require a very dense map containing a large number of SNPs. Although this seems daunting, the rapid progress of the Human Genome Project and the current hunt for SNPs, combined with advances in technology, make this large number attainable. Finding the susceptibility genes and characterizing the biochemical consequences of the genetic variants associated with mental illnesses will improve the treatment and management of these disorders. In the long run, these discoveries may even point to potential cures.

Kruglyak L: Prospects for whole-genome linkage disequilibrium mapping of common disease genes. Nature Genetics 22: 139-144, 1999.

TelemedicineBQuality Health Care at a Distance

Background: Telemedicine is the use of telecommunications technology for medical diagnosis and patient care, and is a medium for delivering medical services to sites that are at a distance from the provider. Telemedicine offers one of the best and most cost-effective opportunities for improving quality and access to health care. Telemedicine includes everything from the use of standard telephone service to high-speed, high-bandwidth transmission of digitized signals in conjunction with computers, fiber optics, satellites, and other sophisticated peripheral equipment and software. Telemedicine has the potential to improve the delivery of health care in rural America and in our inner cities. Telemedicine has particular promise for the provision of home care to the elderly and chronically ill. Remote visiting nurses can reach homebound patients and avoid more costly interventions and premature institutionalization of these patients.

Advance: NIH is supporting projects that are evaluating the use of telemedicine in a wide variety of settings, from the care of newborns and children with disabilities, to the elderly and chronically ill, and those needing a range of specialist care. These projects are helping us determine how we can best use information technology for clinical decision-making. Applications include testbed networks to share information resources, computerized patient records, and medical images; telemedicine projects to provide consultation and medical care to patients in rural areas; and advanced computer simulations of human anatomy for training via "virtual surgery." The projects serve as models for evaluating the impact of telemedicine on cost, quality, and access to health care; assessing various approaches to ensuring the confidentiality of health data transmitted via electronic networks; and testing emerging health data standards.

Implications: Telemedicine research is an important step in developing new computing and communications technologies to improve the quality of the Nation's health care. For example, the new technology will allow a doctor in a rural area to send X-ray images and other medical information instantly to specialists at a faraway medical center for a second opinion. The use of telemedicine not only has the potential to improve health care delivery, but also to contain costs through sharing scarce resources. Telecommunications applications such as computerized patient records could reduce health care costs by $36 billion to $100 billion each year, while improving quality and increasing access. By using telemedicine, doctors and other health care providers can consult with specialists thousands of miles away, continually upgrade their education and skills, and share medical records and X-rays.

http://www.nlm.nih.gov/research/telemedinit.html

Imaging Live Embryos with Two-Photon Microscopy

Background: Although confocal microscopes allow visualization of live specimens, the high-energy light (photons) needed to excite fluorescent probes often damages living cells and makes prolonged observation impossible. An imaging technique that enables study of functions in living cells while preventing photon-induced injury would be a significant improvement.

Advance: Most confocal microscopes use single, high-energy photons to excite fluorescent probes in specimens. But an alternative, relatively new technique known as two-photon microscopyBinvented and developed by Cornell University scientistsBlimits photodamage by using two or more low-energy infrared photons to simultaneously excite fluorescent probes. This lower energy light, coupled with the limited focal plane of the microscope, causes significantly less photo bleaching than conventional single-photon fluorescent imaging. Thus, living specimens can be imaged over hours or even days. To evaluate the effectiveness of the two-photon approach, researchers at the Integrated Microscopy Resource at the University of Wisconsin, Madison, viewed hamster embryos under the microscope, reimplanted the embryos into animals, and generated live offspring with no apparent developmental defects.

Implications: Two-photon microscopy allows previously unobtainable observations of functioning cells. But because the laser technology required for this microscopy approach is often prohibitively expensive, two-photon microscopy is available in only a few facilities nationwide. Fortunately, recent breakthroughs in laser fabrication promise to significantly reduce the cost of these devices. In addition to viewing molecular activity in living cells, two-photon microscopy may facilitate the tracking of cell migration and gene expression during embryonic development and may even allow cancer researchers and clinicians to study cancer cell metastases through living tissue.

Squirrell JM, Wokosin DL, White JG, and Bavister BD: Long-term two-photon fluorescence imaging of mammalian embryos without compromising viability. Nature Biotechnology 17:763-7, 1999.

Rapid, Comprehensive Analysis of Protein Complexes

Background: Many important cellular processes are performed and regulated within macromolecular complexes such as the ribosome, a protein-manufacturing "factory" that is itself made up of numerous unique proteins. Conventional genetic and biochemical methods for analyzing the components of macromolecular complexes generally focus on one target gene or protein at a time. Even when macromolecular complexes are isolated from an intact cell, the protein components are separated and identified individually. Methods to identify complex mixtures of proteins without the need to purify each component to homogeneity would not only improve the efficiency of protein identification, but also increase the sensitivity of detection.

Advance: Investigators have developed a rapid, selective process called DALPC (direct analysis of large protein complexes) that is capable of comprehensively identifying individual proteins in even the most complex macromolecular complex in the cell without first purifying each protein component. This novel approach couples multidimensional liquid chromatography and tandem mass spectrometry with an automated comparison of mass spectra and translated genomic sequences. Assisted by the yeast genome resource at the University of Washington, Seattle, the scientists used DALPC to analyze a ribosome of the yeast *Saccharomyces cerevisiae.* In a single run-through, DALPC identified 75 of 78 known ribosomal proteins, most of which had been previously identified by more labor-intensive methods. The technique also detected a previously unknown ribosomal protein. The scientists analyzed the novel yeast protein and found it to be similar to a poorly understood human protein known as RACK1, which the researchers subsequently discovered is present in human ribosomes.

Implications: Previously, it would have taken years for a team of biochemists to analyze a large multiprotein complex like the ribosome. Directly identifying proteins from complexes bypasses the potential limitation of gel electrophoresis, including protein insolubility and limited fractionation ranges. The DALPC is highly automated and enables rapid, repeated analysis of protein complexes. This new method will provide crucial insights into complex biological phenomena and will greatly contribute to biological investigations in the post-genomic era.

Link AJ, Eng J, Schieltz DM, et al.: Analysis of protein complexes using mass spectrometry. Nature Biotechnology 17:676-82, 1999.

Magnetic Resonance Imaging of Cartilage May Aid Early Diagnosis, Treatment of Osteoarthritis

Background: Osteoarthritis, a major cause of disability in the over-50 population, affects more than 40 million Americans and imposes considerable expense on the health care system. There is no known cure for this debilitating disease, and current treatments focus only on symptomatic relief. A major hindrance to the study and treatment of osteoarthritis has been the lack of reliable methods for early detection of disease and for monitoring disease progression and response to treatment.

Advances: To enable early diagnosis of osteoarthritis, scientists at a magnetic resonance imaging (MRI) resource at the University of Pennsylvania are developing noninvasive techniques for detecting subtle degenerative changes in cartilage, which is the primary pathology associated with the disease. The researchers used a new sodium MRI technique to image both healthy bovine cartilage and cartilage that had been partially degraded by the enzyme trypsin, which mimicked the cartilage deterioration seen in osteoarthritis. The new imaging technique revealed several unique properties of the degraded cartilage, including increased permeability and reduced elasticity. The imaging approach lends insight to the design of future in vivo experiments.

Implications: These promising new imaging methods may one day enhance physicians' abilities to diagnose osteoarthritis, intervene with appropriate therapeutics, monitor clinical outcomes, and evaluate potential new therapies, including cartilage-protecting drugs and gene therapies. [secondary B treatment and diagnosis]

Kaufman JH, Regatte RR, Bolinger L, Kneeland JB, Reddy R, and Leigh JS: A novel approach to observing articular cartilage deformation in vitro via magnetic resonance imaging. Journal of Magnetic Resonance 9:653-62, 1999.

New Models for Migration Patterns into the United States

Background: Census Bureau projections of immigrants coming to the United States from Mexico and other countries have consistently underestimated the actual numbers and composition of those who have come across our borders. In part, this chronic underestimation has been due to the difficulty of monitoring the movements of immigrants, both legal and illegal. As a result, demographers and policymakers have been forced to rely on projections based on the best information available, however outdated. These inaccuracies have had the most impact in those states that are principal immigrant-receiving states: California, New York, Florida, Texas, and Illinois.

Advance: Using advanced modeling techniques developed over the last few years, researchers in the United States and Mexico have constructed a dynamic model that more accurately estimates the numbers of immigrants who move back and forth across the U.S.-Mexico border. These improved estimates are the result of understanding the movement patterns of immigrant families, as opposed to the movements of immigrants acting alone. Additionally, researchers now have a better understanding of the psychological motivations behind people=s decisions to move to another country, further improving the model. As a result, when compared with the Census Bureau projections, researchers have shown that previous estimates significantly underestimated the actual net migration, with almost twice as many people coming into the United States as earlier estimated.

Implication: The use of new modeling techniques to predict more accurately population increases and immigrant movement, particularly in areas with high immigration, will have tremendous implications for researchers, health care providers, and policymakers in the years to come.

Massey DS and Zenteno RM. The Dynamics of Mass Migration. Proceedings of the National Academy of Sciences 96: 5328-5335, 1999

DNA Sequencing Technology: Faster, Better, Cheaper

Background: The chemistry that is most commonly used today for DNA sequencing was invented by Fred Sanger in the mid-1970s. At that time, sequencing of 1000 bases was a laborious process that required the efforts of a highly trained professional biologist for many months. An automated DNA sequencing device was invented in the mid 1980s, and commercialized in the early 1990s. That machine evolved incrementally through the decade, as usability, reproducibility, data quality, and throughput improved. However, these machines require the pouring and loading of slab gels, both time and labor intensive processes. The next generation of DNA sequencing machines/technology should be more automated and more efficient to increase speed and decrease cost.

Advance: The realization of high throughput (production) sequencing using automated capillary array electrophoresis is a critical and exciting advance in DNA sequencing technology. In 1999, this technology, originally developed in U.S. and Canadian university laboratories, has been developed into commercial instruments for widespread use by two different instrument companies. These instruments will sequence DNA much more rapidly, while producing nearly the same data quality, with much less human intervention (due to automation) than has been required for sequencing with the previous generation of semi-automated slab gel instruments. The majority of the human genome sequence is now being determined using these machines.

Just as capillary array sequencers are currently replacing slab gel machines, the capillary systems are likely to be supplanted in three to five years by a new generation of microfabricated array sequencers that will offer additional advantages. Microfabricated systems, including those being developed by NIH-funded scientists, will potentially be very compact (a consideration for laboratories conducting thousands of DNA sequencing experiments) and separate DNA as fast or faster than capillaries. These devices offer the potential to reduce by 10- or 100-fold the amount of DNA sample needed for the experiments, thereby greatly reducing the cost of supplies.

Researchers are now developing a further improvement in sequencing technology that integrates sample preparation and DNA sequencing into a single machine. Scientists at the University of Michigan have designed a prototype device that can mix DNA samples and reagents, performing the sequence reaction and detection on a small microfabricated chip. When manufactured at large scale, such devices would be extremely inexpensive and could be used to accomplish a wide array of DNA tests in a simple Asample-in-results-out@ (hands-off) format.

Implications: Advances in DNA sequencing technology have contributed significantly to the increased efficiency and decreased cost of sequencing the human genome. But bringing new sequencing technology into use will continue to be important even after the human genome has been sequenced. In fact, scientists= appetites for sequence data will only increase. Sequencing will be needed to decipher the genomes of other organisms and to study human genetic variation. Continued support for DNA sequencing technology development will generate incremental improvements that can be implemented within the next few years as well as totally revolutionary technologies that may not be implemented for 10 years but that could increase the speed and decrease the cost of sequencing by 10-100 fold. Intense collaborations between technology

developers and experienced large-scale DNA sequencing laboratories are being encouraged and supported to hasten the movement of new technologies from demonstration to implementation.

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Schmalzing D, Adourian A, Koutny L, Ziaugra L, Matsudaira P, and Ehrlich DJ: DNA sequencing on microfabricated electrophoretic devices, Analytical Chemistry 70: 2303-10, 1998.

Burns MA, Johnson BN, Brahmasandra SN, Handique K, Webster JR, Krishnan M, Sammarco TS, Man PM, Jones D, Heldsinger D, Mastrangelo CH, and Burke DT: An integrated nanoliter DNA analysis device. Science 282: 484-87, 1998.

SNPs: New Tools for Tracing Inherited Diseases

Background: Any two people have the same DNA sequence at about 99.9% of their DNA. The 0.1% difference represents genetic variation that can lead to differences in the risk of getting various diseases. Some diseases, such as cystic fibrosis and Huntington=s disease, result from differences in DNA sequence in single genes. However, many common diseases such as diabetes, cancer, heart disease, psychiatric disorders, and asthma are influenced by complex interactions between multiple genes as well as by non-genetic factors such as diet, exercise, smoking, and exposure to toxins. With the tools of the Human Genome Project, finding the genes for diseases caused by alterations in single genes has become relatively straightforward. Finding the genes that contribute to common diseases remains extremely difficult. A catalogue of the places in the genome where the DNA sequence differs among individuals will help in the effort to discern the genetic signals associated with a disease amid the noise from other influences on the disease.

Advance: The NIH organized the establishment of the DNA Polymorphism Discovery Resource (PDR). It consists of 450 DNA samples collected under strict ethical guidelines from anonymous unrelated United States residents of diverse ethnic backgrounds. The PDR is now the major resource being used to look for DNA variants known as single nucleotide polymorphisms, or SNPs. The NIH has funded studies to allow researchers to look for SNPs in a common set of samples, thereby making it easier to identify inherited disease risks. Some research groups are looking for SNPs in genes that are related to particular diseases; others are looking for variants throughout the human genome. In the next two years, researchers expect to find about 400,000 SNPs. This effort is complemented by the SNPs Consortium, the collaborative effort of 10 large pharmaceutical companies and the Wellcome Trust to identify 300,000 SNPs.

Implications: Armed with a robust catalogue of SNPs, researchers can then study people with and without particular diseases to figure out which variants are related to differences in disease risk and response to therapies. The large number of genetic variants will help researchers identify disease-related genes, especially for common diseases, with the goal of understanding the underlying causes of the diseases. Variants found to be related to a disease will facilitate development of diagnostic tests and provide targets for further study to understand the biological processes underlying health and disease. This understanding in turn will fuel development of improved prevention and treatment strategies. As genetic variants contribute to individual differences in response to drugs, the identification and understanding of these variants will allow doctors to choose the most effective drug based on a patient's particular variants. [secondary B diagnosis]

Collins FS, Brooks LD, and Chakravarti A: A DNA polymorphism discovery resource for research on human genetic variation. Genome Research 8: 1229-1231, 1998.

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Halushka MK, Fan J-B, Bentley K, Hsie L, Shen N, A Weder, Cooper R, Lipshutz R, Chakravarti A: Patterns of single-nucleotide polymorphisms in candidate genes for blood-pressure homeostasis. Nature Genetics 22:239-47, 1999.

The Complete Sequence of the Yeast Genome: Simplifying the Study of Complex Biological Processes

Background: The budding yeast, *Saccharomyces cerevisiae*, is an important model organism to study biological properties of cells that contain a nucleus. A significant advance in the study of this organism was the completion of the entire genomic sequence in 1996. The major challenge that remains is determining the function of the approximately 6000 genes encoded in the genome. Although much biological research has been conducted on *S. cerevisiae* over the past decades, the function of a large fraction of these genes is not known.

Historically, functional analyses were conducted one gene at a time. Knowledge of the complete genome now allows for the analysis of genome function on a global scale, through the use of systematic and comprehensive strategies. Indeed, over the past several years, a number of new technologies to implement such strategies have emerged. These technologies include various methods for analyzing gene expression (at both the RNA and protein level), generating and analyzing mutations that alter gene expression, detecting which proteins interact with one another, and determining the cellular localization of proteins.

Advance: The availability of the complete DNA sequence of the yeast genome, together with new DNA microarray technologies, have armed researchers with powerful tools to study complex biological processes. Scientists have made microarrays consisting of DNA from nearly all of the yeast genes. These microarrays allow for highly rapid, efficient and comprehensive studies since scientists can ask questions about all the genes in one experiment rather than one gene at a time. Research with yeast microarrays is producing vast amounts of valuable data. For example, yeast microarrays have been used to monitor gene expression to gain insight into complex biological processes, such as metabolism, gene regulation, sporulation (sexual reproduction) and evolution. These methods allow the identification of the relationship between genes involved in or affected by specific biological processes.

DNA microarrays are also being used to study the function of yeast genes. By creating a mutation in the gene and thereby eliminating its function, researchers can observe the effect and gain clues into the function normally provided by the gene. With the entire genome in hand, researchers generated a comprehensive set of mutations in which a molecular tag or Abarcode@ was inserted into 2026 different genes in the yeast genome. These barcode insertions resulted in the precise deletion of the targeted genes and DNA microarray technology enabled the researchers to simultaneously examine the characteristics of the 2026 mutant yeast strains.

Implications: The combination of the availability of the complete genome sequence together with new genomic technologies is spurring research in which the complete set of genes of an organism can be studied and analyzed in a highly parallel, rapid fashion. The amount of data generated from a single such experiment dwarfs the amount of data generated by years of genetic research using conventional methods. New genomic data are providing clues to gene function, gene regulation, pathways and networks. This body of knowledge is greatly increasing our understanding of yeast biology and is serving as a test-bed to refine the methods so that they can be applied to other organisms as their entire genomic sequences emerge. Because genes in yeast

and mammals encode very similar proteins, genomic analysis in yeast provides a wealth of data about genes in humans, including many genes implicated in disease.

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Ferea TL, Botstein D, Brown PO, and Rosenzweig RF: Systematic changes in gene expression patterns following adaptive evolution in yeast. PNAS 96:9721-26, 1999.

Winzeler EA et al: Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. Science 285:901-06, 1999.

Sequencing the Human Genome, Our Genetic Instruction Book

Background: The Human Genome Project (HGP) started in 1990 and, from its beginning, has enjoyed significant success. Several of the project's initial goals have been achieved, including building maps to localize and order the position of genes in both the human and mouse genomes, and sequencing the genomes of the E. coli bacteria, bakers yeast, and the C. elegans roundworm. In addition, sequencing the genome of the fruit fly (*Drosophila melanogaster*) is nearly complete. The ability to compare the sequence of genes across multiple species and develop model systems in simpler organisms will significantly enhance the ability of researchers to identify the functional roles of the encoded proteins. In FY96, in preparation for tackling the much larger and more complex human genome, pilot projects began to test new technologies and strategies for sequencing. Eight scientific teams supported by NIH, the Department of Energy (DOE) and international collaborators had completed the sequence of over 480 million bases by March 1999, of which 260 million (or close to 10 percent of the human genome) were in high-quality finished form. The pilot projects produced finished sequence that met or exceeded the international accuracy standard of no more than 1 error in 10,000 bases and drove down the cost of sequencing to an average of 20 - 30 cents per base.

Based on the success of the pilot phase, in March 1999, an international consortium launched the full-scale effort to sequence the estimated 3 billion basepairs that make up the human genetic instruction book. The consortium, with the U.S. taking the lead, and with important participation by the U.K., Germany, Japan and China, expects to produce at least 90 percent of the human genome sequence in a Aworking draft@ form by the spring of 2000, years earlier than expected.

Advance: In November 1999, the consortium completed the sequence of one billion of the approximately 3 billion bases in the human genome. Achieving this important milestone marks the success of the transition from the pilot to the full-scale production sequencing. The consortium, a model of international scientific collaboration led by the NIH, the DOE, and the Wellcome Trust, is on track to complete the working draft in the spring of 2000 and the final, high quality genome sequence by 2003 or earlier.

All sequence data produced by the international consortium is deposited in public databases for free access within 24 hours. The rapid public availability of the sequence is invaluable to corporate researchers engaged in drug development, as well as academic scientists studying the molecular basis of human health and disease.

Implications: Determining the complete genetic blueprint of the human will greatly accelerate the identification of the genes underlying many human diseases, including complex diseases that represent the greatest health burden to the U.S. population. Identifying those genes is the first step to a more profound understanding of the biological basis of disease and this, in turn, will lead to much more effective and inexpensive ways to diagnosis, treat and prevent disease.

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http://www.ncbi.nlm.nih.gov/genome/seq/

Chromosome Healing in Embryonic Stem Cells

Background: Human genetic material is organized into 46 chromosomes, the tips of which are called telomeres. During normal cell division the tips of the chromosomes shorten, leading to an inability of the cells to further propagate. Unlike most cells in the human body that have a limited ability to replicate, stem cells can divide infinitely by healing the ends of their chromosomes. While some studies in cancer cells have shown that an enzyme called telomerase heals the ends of chromosomes, it is not clear if normal stem cells use the same mechanism.

Advance: Very often discoveries in science are linked to the development of new technologies or assays. A research team has developed a novel assay for the analysis of chromosome healing. This assay is based on targeted double-strand DNA breaks near the end of the chromosome and unique selection systems to discern the molecular steps in the process. This research team found that the chromosome telomers can be healed in the apparent absence of the action of telomerase through double-strand break repair.

Implications: Many cell types in the human body, such as neurons or muscle cells have lost the ability to replicate. Thus loss of a significant population of a particular cell-type can lead to specific human pathologies. For example the loss of dopaminergic neurons in the substantia nigra is associated with Parkinson-s Disease. Stem-cells, cells which have the ability to replicate, hold great promise in the treatment of specific diseases associated with a loss of a particular cell type, such Parkinson-s Disease. Understanding how stem-cells keep their chromosome tips from shortening is fundamental to the treatment of specific diseases. These studies have helped develop the necessary tools to investigate the precise molecular events by which stem-cells heal their chromosome ends.

Sprung CN, Sabatier L, Murnane JP: Telomere dynamics in a human cancer cell line. Exp. Cell. Res. 247:29-37, 1999.

Sprung CN, Reynolds GE, Jasin M, Murnane JP: Chromosome healing in mouse embryonic stem cells. Proc. Natl. Acad. Sci. U. S. A . 96:6781-6786, 1999

Alcoholic Women Suffer Greater Brain Loss

Background: Alcoholism traditionally has been regarded as a man=s disease, but almost 4 million U.S. women suffer from alcoholism or abuse alcohol. Researchers are in the beginning stages of identifying biological differences in how men and women respond to alcohol. Studies suggest that women who have been heavy drinkers for fewer years than heavily drinking men sustain equal amounts of brain damage. Women who drink proportionally the same dose of alcohol as men reach higher blood-alcohol levels. Alcoholic women are more vulnerable than are alcoholic men to liver and heart damage.

In this study, researchers asked if the hippocampus, an area of the brain involved in memory, emotion, and cognition (knowing, awareness, and judgment), is more vulnerable to alcohol-induced damage in the female brain than in the male brain. They chose the hippocampus because (1) changes in memory are among the earliest in alcoholism; (2) the hippocampi of alcoholics are smaller than those of healthy people; (3) the hippocampus is rich in receptors for stress hormones, which increase with alcohol consumption and can cause damage when produced in excess; and (4) alcoholic women have a high rate of post-traumatic stress disorder (PTSD), and some studies suggest that the hippocampus is smaller in people with PTSD.

To test their hypothesis, researchers used magnetic resonance imaging (MRI) to measure the volumes of space that various structures of the brain occupy in alcoholic and nonalcoholic men and women. By using adjusted mathematical calculations to guide the MRI computers and to interpret the data they generated, researchers achieved more accurate hippocampal measurements than previously were documented.

Advance: As expected, the researchers found that the hippocampus was smaller in the alcoholics than in the nonalcoholics. They also found that this reduction was greater in alcoholic women than in alcoholic men. However, contrary to what researchers expected, the ratio of the volume of the hippocampus to the volume of the rest of the brain was the same in nonalcoholics and in alcoholics. In other words, there was no evidence that the hippocampus was selectively affected in alcoholism. In addition, PTSD did not affect hippocampal volume, contrary to previous reports.

Implications: With this study, researchers extended the map that shows what structures of the brain are vulnerable to alcohol damage. Having identified these areas, scientists can (1) correlate them with changes in functional ability in alcoholics, enabling investigators to focus on areas of brain damage that have the most impact and (2) explore these areas at the molecular level, to look for the biological mechanisms by which alcohol damages them. Once researchers identify these damaging mechanisms, they become potential targets for pharmaceutical interventions. The differences in male and female brains described here will be useful to various fields of research. These findings also suggest that researchers from all disciplines take into account their subjects= history of alcohol consumption when studying hippocampal damage.

Agartz I, Momenan R, Rawlings RR, Kerich MJ, Hommer DW. Hippocampal Volume in Patients with Alcohol Dependence. Archives of General Psychiatry, 56(4):356-363.

Visualizing the Activity of Respiratory Pacemaker Cells in the Mammalian Brain

Background: Breathing disorders during sleep are significant public health problems, particularly Asleep apneas@Bthe temporary cessation of breathing in adultsBand the more severe arrest that may cause sudden infant death syndrome (SIDS). These disorders may result in part from unstable activity of the respiratory pacemaker, a group of nerve cells in the base of the brain that generates the rhythm of breathing, as the brain enters the deep stages of sleep. Progress in understanding the brain activity that underlies normal and abnormal breathing has been hampered by the difficulty in identifying and studying the activity of individual pacemaker cells.

Advance: New methods now enable scientists to directly visualize the activity of respiratory pacemaker cells in living mammalian brain tissue removed from an animal=s body and kept alive under carefully controlled conditions. Scientists can label pacemaker cells with dye molecules that emit light when the cells become electrically active, thus identifying the cells involved in breathing. Then, guided by microscopic observation, researchers attach fine glass electrodes to individual cells and use sophisticated electronics to study the electrical behavior of the identified pacemaker nerve cells that control breathing.

Implications: Understanding how breathing is normally controlled at the level of individual nerve cells is essential for understanding conditions that can cause life-threatening cessation of breathing, particularly during sleep. These techniques can be used to follow changes in the control of breathing in neonatal and juvenile animals that might provide clues to the causes of SIDS as well as to a broader understanding of all forms of apneas that occur because of abnormal pacemaker function.

Koshiya N and Smith JC: Neuronal pacemaker for breathing visualized *in vitro*. Nature 400:360-63, 1999.

Enhanced Threading Method for Protein Structure Prediction

Background: The determination of the three-dimensional structure of a protein from its amino acid sequence alone may be considered to be the "holy-grail" of present day biochemistry. The solution of this problem is of such great importance because it would amplify the information now present in the nucleic acid sequence databases by allowing protein structure and function to be inferred directly from a gene sequence. At present, the determination of a single protein structure using experimental techniques is laborious and time-consuming.

Every few years, the protein science community hosts a competition designed to assess the state of the art in protein structure prediction. Three such competitions have been held to date, the most recent of which was Third Meeting on the Critical Assessment of Techniques for Protein Structure Prediction (CASP3). At CASP3, 98 entrants collectively submitted a total of 3,807 structure predictions for a set of 43 target proteins whose structures had been determined experimentally, but not revealed.

Advance: The National Center for Biotechnology Information (NCBI) structure group, headed by Steve Bryant, was ranked first in the fold-recognition category in CASP3. The predictions of the NCBI team were made entirely on the basis of the amino acid sequences of the target proteins. The prediction technique involved the Athreading@ of a target protein sequence through the three-dimensional structure of another protein, called a template. The template was selected from the database of known structures using fold-recognition algorithms. Hypothetical Athreadings@ of the target sequence through the structural template were scored using conventional methods enhanced with the aid of a Position Specific Scoring Matrix (PSSM) generated using PSI-BLAST, a database search tool developed at NCBI.

Implications: With the sequences of most of the genes in the human genome expected within the next year, the ability to reliably model the protein products of these genes could quickly yield thousands of models for protein structures which would take years to determine by conventional techniques, such as X-ray crystallography. Any of these model structures might well prove to be the key to deciphering the cause of a human disease. The value of the sequences of human genes determined through the Human Genome Project will be greatly magnified to the extent that protein structures can be predicted from these sequences.

Panchenko, A, A Marchler-Bauer, and SH Bryant. Threading with explicit models for evolutionary conservation of structure and sequence. *Proteins* Suppl 3 (1999). In press.

CASP3 Web site: http://predictioncenter.llnl.gov/casp3/

GeneMap98 Provides a Scaffold for Human Genome Project Data by Mapping 30,000 Human Genes

Background: An international consortium was formed in 1994 to construct a gene-based map of the Human Genome by determining the locations of expressed sequence tags (ESTs) relative to a framework of well-characterized genetic markers. The first map to result from this effort was the Gene Map of the Human Genome, which appeared in 1996 and included 16,354 gene-based markers. The successor to this 1996 transcript map, GeneMap98 and the GeneMap99 update, feature more than 30,000 loci, or about half of the 60,000 to 80,000 genes thought to be contained in the human genome.

Advance: The process of constructing GeneMap98 involved the mapping of short (typically, only a few hundred base pairs), unique DNA sequences, termed sequence tagged sites (STSs), within a framework of well-characterized genetic markers developed by the Genethon Corporation. The technique used to locate the STSs within the framework of genetic markers is known as radiation hybrid (RH) mapping.

Radiation hybrid mapping employs a panel of human-on-hamster hybrid cell lines, each containing its native hamster genome as well as a random assortment of human chromosomal fragments. These chromosomal fragments are produced by X-ray irradiation of human genomic DNA to produce random, radiation-induced breaks in the chromosomes. Two such hybrid cell panels have been employed to create GeneMap98: the GeneBridge 4 (GB4) panel and the Stanford G3. The two RH panels complement one another in that GB4 provides greater long-range continuity, whereas G3 provides increased map resolution.

The location of an STS is determined by performing a set of polymerase chain reaction (PCR) assays using the primer pair associated with the STS in question and the DNA isolated from each of the various cell lines constituting a radiation hybrid panel. If the target STS is present within the human DNA fragments isolated from a particular cell line, a PCR product of the correct length is observed. In this case, the marker is said to be "retained" within the DNA of the cell line yielding the correct PCR product. The STS is mapped for placement on GeneMap98 by performing a statistical comparison of its pattern of retention within the cell lines constituting the RH panel with the retention patterns arising from other STSs.

Implications: A complete human gene map will be essential to progress toward a deeper understanding of human biology and disease. GeneMap98 will provide a scaffold on which to mount the large-scale sequencing data being generated daily as the sequencing of the human genome progresses. The map will also accelerate the pace of the discovery of human disease genes by positional cloning. In the year following the publication the 1996 Gene Map of the Human Genome, for instance, the isolation of 16 genes by positional cloning techniques was reported. Of these 16 genes, 7 had already been isolated as ESTs and mapped at the time of their cloning. GeneMap99 now includes mapping information for 11 of these 16 genes.

Deloukas, P, et al. A physical map of 30,000 human genes. *Science* 744_6, 1998.

HIV-1 Subtyping Tool Simplifies the Detection of Mosaic HIV-1 Genomes

Background: HIV-1, the retrovirus responsible for the AIDS pandemic, has a highly variable RNA genome that has diversified into multiple subtypes. The genetic diversity of HIV-1 is exemplified by the fact that members of the two main genetic groups, group M (major) and group O (outlier), differ by up to 47% in the amino acid sequences of their envelope proteins. Within group M, HIV-1 can be subdivided into at least nine distinct subtypes, among which there is a 25 to 35% variation in the amino acid sequence of the envelope protein. The genetic variability of HIV-1 is not limited to the envelope protein but is also seen in sequences throughout the HIV-1 genome.

Advance: To facilitate the monitoring of HIV-1 genomes, the National Center for Biotechnology Information (NCBI) has developed a Web-based subtyping system. The subtyping method employs a blastn (one variant of NCBI=s versatile BLAST sequence-similarity search suite) comparison between the HIV-1 sequence to be subtyped and a panel of reference sequences taken from the principal HIV-1 variants. The subtyping panel includes complete genomic references for the A, B, C, D, E, F, G, and H group M subtypes as well as for group O and the recently described N sequence. During the subtyping process, multiple blastn comparisons are made over a sliding window of a size and step value set by the user. A color-coded graph of the blastn sequence similarity score against window location is generated for comparisons between the query sequence and each reference sequence in the panel.

Implications: The genetic diversity of HIV-1 presents a problem in vaccine development. Most vaccines currently being tested are most effective against HIV-1 subtype B; however, as other subtypes develop and spread, vaccines specific to these variants will be required. The fact that a significant fraction of HIV-1 isolates have mosaic genomes resulting from inter-subtype recombination further complicates the problem of vaccine development and highlights the need for subtype monitoring. Mosaic HIV-1 genomes can be detected easily using the NCBI HIV-1 subtyping system. This will make it possible to track the metamorphosis of HIV-1 as it spreads and to detect novel, and potentially dangerous, new strains more easily.

HIV-1 Subtyping Tool Web site: http://www.ncbi.nlm.nih.gov/retroviruses/HIV1/

dbSNP: A Database of Single Nucleotide Polymorphisms

Background: Single nucleotide polymorphisms (SNPs) are the most common variations in the human genome, occurring once every 100 to 300 base pairs. Because of the volume of data represented by SNPs, a database of SNPs is expected to greatly facilitate large-scale associative genetics studies concerned with the linkage between sequence variation and heritable phenotypes.

Advance: The NIH, in collaboration with the National Center for Biotechnology Information (NCBI), has established dbSNP as a central, public, repository for SNP data as well as for data on short insertions or deletions. The database includes information on sequence variations within individuals and populations as well as descriptions of the assay conditions used to detect each variant. Systems for the integration of dbSNP with other genomic data at NCBI, including GenBank, are also under development.

Implications: The study of SNPs is expected to accelerate the identification of disease genes through the associations between diseases and particular SNPs in a human population. This SNP-based approach is more rapid than the typical approach of pedigree analysis, which tracks transmission of a disease through a family, because it is much easier to obtain DNA samples from a random set of individuals in a population than it is to obtain them from every member of a family over several generations. NCBI-s dbSNP will provide a valuable resource in the hunt for disease-causing mutations by organizing SNP sequence data and integrating this data with information on SNP frequencies and procedures for SNP detection.

Sherry, ST, M Ward, and K Sirotkin. dbSNP_Database for single nucleotide polymorphisms and other classes of minor genetic variation. *Genome Res* 9(8):91-8, 1999.

dbSNP Web site: http://www.ncbi.nlm.nih.gov/SNP/

HIV Alters the Kinetics of T cells

Background: In the human body, where millions of cells are born or die every second, it is a formidable task to evaluate which cells are healthy and multiplying and which are languishing and dying in different stages of health and disease. But recognizing and reacting to these cellular phenomena with proper treatment can help save the lives of patients with diseases such as AIDS, in which the human immunodeficiency virus (HIV) depletes the immune system of certain essential cells. Cell proliferation is sometimes measured in vitro or in animals using radioactive thymidine or brominated deoxyuridine, but these compounds are considered too toxic for human use. As a consequence, scientists have resorted to indirect methods for quantitating this important parameter in research subjects.

Advance: Researchers have developed a safe technique for directly monitoring the body's production of immune cells through use of deuterium-labeled glucose incorporated into DNA. This non-radioactive isotope is detectable by using gas chromatography/mass spectroscopy techniques and poses no hazard when injected into human subjects. By measuring the quantities of deuterium-labeled purine deoxynucleosides present in DNA extracted from test cells, the investigators have shown that this methodology replicates the results obtained from the hazardous compounds used in current in vitro studies. Using this new methodology to evaluate HIV-infected patients at the University of California, San Francisco, the investigators demonstrated that HIV infection shortens the life span of CD4+ and CD8+ T cells by two-thirds without a compensatory increase in T-cell production rates. This effect is reversed by highly active anti-retroviral therapy.

Implications: These observations contradict the popularly held belief that HIV kills infected T cells and induces a transient compensatory increase in T cell production, which eventually exhausts the immune system and causes death. The new findings also suggest that stimulating T cell growth may represent another means by which AIDS can be treated. [secondary B treatment]

Hellerstein M, Hanley MB, Cesar D, Siler S, Papageorgopoulos C, Wieder E, et al.: Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. Nature Medicine 5:83-9, 1999.

SCIENCE CAPSULES

Ultra-Small Porous Materials Synthesized. NIH-supported researchers have discovered a method for creating a new class of porous materials with an orderly crystal-like arrangement of ultra-small spherical spaces. There are many potential uses for these materials in both basic research and the pharmaceutical industry. For example, minute compartments capable of holding individual enzyme molecules could be produced using these materials, providing the potential for the development of more stable and longer-lasting sensors like glucose sensors used in the treatment of diabetes mellitus.

Johnson SA, Ollivier PJ, and Mallouk TE: Ordered mesoporous polymers of tunable pore size from colloidal silica templates. Science 283: 963-965, 1999.

Keeping Track of Memory T Cells. Memory T cells, cells that persist after infection or immunization, enable the immune system to mount an enhanced response to invaders that the organism has previously encountered. It has been difficult to study immunologic memory because memory T cells lack natural markers that are specific and permanent. Researchers have utilized their knowledge of genetic mechanisms to develop a technique for marking memory T cells in miceBan achievement potentially useful for vaccine development and for studies of natural immunity, transplantation, immunodeficiencies, and autoimmune diseases.

Jacob J and Baltimore D: Modeling T-cell memory by genetic marking of memory T cells in vivo. Nature 399: 593-597, 1999.

Measuring Quality of Medical Care. Academic researchers and industry representatives collaborated with NIH to develop a classification system for rehabilitation patients that could recognize differences in quality of care while monitoring costs. This first such research-based classification system is based upon length of stay and outcome measures related to functional independence. The classification system should help improve health care quality for seniors, children, and others with disabling conditions by providing alternative payment modules for inpatient rehabilitation services.

Stineman MG: Function-related groups 101: a primer. Crit Rev Phy & Rehab Med 10: 319-358, 1998.

Stineman MG, Ross RN, Williams SV, Goin JE, and Granger CV: A functional diagnostic complexity index for rehabilitation medicine: measuring the influence of many diagnoses on functional independence and resource use. Arch Phys Med Rehabil; In press.

New Respiratory Muscle Endurance Test Less Stressful, Potentially More Accurate. Measuring the endurance of the respiratory muscles in patients with chronic obstructive pulmonary disease (COPD) is a standard means of measuring disease progression and response to therapy. Scientists have devised a new method of measuring muscle endurance using

discontinuous incremental threshold loading, which is less strenuous and stressful to the patient and is more reliable. This study has the potential to change clinical practice with COPD patients.

Larson JL, Covey MK, Berry J, Wirtz S, Alex CG, Matsuo M: discontinuous incremental threshold loading test: measure of respiratory muscle endurance in patients with COPD. Chest 115:60-67, 1999.

Growing Evidence of New Bypass Options. By proving the safety of a gene-therapy approach to mitigating impaired circulation in the heart, investigators have taken a major step toward developing less invasive approaches to treating coronary heart disease. Participating patients received a direct injection into the affected heart areas of a virus that was modified to cause production in the heart tissue of a protein, vascular endothelial growth factor (VEGF), which stimulates the formation of blood vessels. Because none of the patients experienced any adverse effects associated with the treatment and all patients showed evidence of clinical improvement, studies of therapeutic effectiveness are now warranted. [secondary B treatment]

Rosengard TK, Lee LY, Patel SR et al: Angiogenesis gene therapy: Phase I assessment of direct intramyocardial administration of an adenovirus vector expressing VEGS121 cDNA to individuals with clinically significant severe coronary artery disease. Circulation 100:468-474, 1999.

New Compound for Studying Brain Receptors for Nicotine May Lead to Better Treatments for Several Diseases. Receptors in the brain where nicotine acts are thought to have significant involvement in nicotine addiction, in cognition, and in several neuropsychiatric diseases, notably Alzheimer=s disease. Researchers have recently produced a new compound that can be safely used to label these receptors in living humans and allow their visualization using brain imaging techniques. This should lead to a greater understanding of how these receptors mediate nicotine=s many effects, which will in turn lead to new ways to promote smoking cessation and to treat cognitive deficits associated with neurodegenerative diseases such as Alzheimer=s disease.

Vaupel DB, Mukhin AG, Kimes AS, Horti AG, Koren AO, London E. In Vivo studies with [125] 5-1-A-85380, a nicotinic acetylcholine receptor radiogland. Neuropharmacology Neuroreport 9: 2311-2317, 1998.

Cortical Cartography for the 21st Century. Efforts to decipher the organization and function of different cortical areasBthat is, topmost layer of the brain responsible for many higher mental functionsBhave traditionally been stymied by the intricately folded topography of the cortex. Now, NIH-funded investigators have developed an integrated suite of software programs for visualizing the cortical sheet and analyzing its structure and function with unprecedented spatial resolution. The new methods already have been used to generate the first surface-based atlas of human cerebral cortex and promise to be invaluable for studying the normal functioning of human cortex as well as the many different neurological diseases and psychiatric disorders that involve abnormalities in cortical structure and/or function.

Drury HA, Van Essen, DC, Corbetta M., Snyder AZ: . Surface-based analyses of the human cerebral cortex. In: Brain Warping, A.Toga et al., eds., Academic Press, pp. 337-363, 1999.

Van Essen DC, Drury HA, Anderson CH: An automated method for accurately reconstructing the cortical surface. NeuroImage 9: S173, 1999.

News clipping: San Francisco Chronicle, November 9, 1998: "Scientists find new ways to map brain" by Carl T. Hall (front page illustration).

Profiles in Science. The *Profiles in Science* Web site is making the archival collections of prominent biomedical scientists available to the public through modern digital technology. The first collection on the site presents the work of Oswald Avery (1877-1955), one of this country's first molecular biologists, whose findings proved that the genetic material is DNA, and the second collection presents the papers of Joshua Lederberg (b. 1925), an American geneticist and microbiologist who received the Nobel prize for "his discoveries concerning genetic recombination and the organization of the genetic material of bacteria". The *Profiles in Science* Web site allows anyone with Internet access to look behind the scenes of scientific findings and share some of the excitement of early scientific discoveries.

http://www.profiles.nlm.nih.gov/

Clinical Trials Database. The Clinical Trials information system is being developed to provide patients, families, and members of the public with easy access to information about clinical research studies, including information about which clinical trials are currently recruiting patients, where the trials are being conducted, what the design and purpose of the research study is, and what the criteria are for participating. An important feature of the database will be to provide access to other online health resources that help place clinical trials in the context of patients' overall medical care. The first version of the system will be available by the end of 1999 and will provide access to NIH-sponsored studies; subsequent versions will include clinical trials data from other Federal Agencies and from private industry.

http://www.lhncbc.nlm.nih.gov/clin/

Tribal Connections. NIH has supported a ground-breaking effort to improve the Internet connectivity on selected American Indian reservations and Alaska Native villages in the Pacific Northwest. By improving connectivity, NIH hopes to facilitate access of Native Americans living in rural, remote areas to health and biomedical information available over the Internet. The NIH project, in collaboration with the Regional Medical Library at the University of Washington in Seattle, is using a community-based infrastructure development approach to help assure that Internet enhancements are responsive to local needs and conditions, and involve the local tribal and village leadership and health community. Twelve tribes and 4 villages in 5 States are currently participating. The project support at each site includes planning and technical assistance, training, and outreach in addition to provision of needed hardware, software, and telecommunications links. NIH is encouraging the involvement of other organizations, such as

the Indian Health Service and the Washington State Library, in order to make best use of scarce resources in a coordinated, sustainable way.

http//:www.tribalconnections.org/

Internet Connectivity Performance Evaluation. An initial evaluation of the performance of end-to-end Internet pathways involving biomedical institutions and users has been performed. The Internet is increasingly used to support biomedical research and scientific collaboration, and thus the quality of Internet performance is of growing concern. This research has developed a set of methods and metrics for assessing Internet performance, and has applied these tools to evaluate the connectivity of Internet pathways between NIH and selected research universities, medical libraries, hospitals, and medical researchers in the U.S. and abroad.

Wood, F.B., V.H. Cid, and E.R. Siegel, "Evaluating Internet End-to-End Performance: Overview of Test Methodology and Results," Journal of the American Medical Informatics Association, Vol. 5, No. 6, Nov/Dec 1998, pp. 528-545.

Measuring Time-Related Changes in Brain Activation. Functional magnetic resonance imaging (fMRI) has revolutionized understanding of how the brain operates. But because fMRI measures brain activation indirectlyBby detecting changes in cerebral blood flow, which lags several seconds behind activation of brain cells themselvesBattempts to measure specific brain activities that unfold over time are often foiled by overlapping signals. But now scientists at the Center for Advanced MR Technology at Stanford University have devised new mathematical and analytical methods that can tease out, or deconvolve, particular fMRI signals produced in response to different stimuli over time, thereby significantly improving temporal resolution and paving the way for further advances in event-related neuroscience.

Glover GH: Deconvulution of impulse response in event related BOLD fMRI. NeuroImage 9:416-429, 1999.

Flow Cytometry Enables Rapid Genome "Fingerprinting." Scientists at the National Flow Cytometry Resource at Los Alamos National Laboratory have demonstrated a sensitive new technique for rapidly analyzing and characterizing bacterial DNA fragments. The new method uses ultra-sensitive flow cytometry to accurately size and analyze DNA segments in a matter of minutes, compared to the hours required for more commonly used analytical techniques. Further development of this technology may enable bacterial identification and subsequent treatment in hours versus days that are currently required. [secondary B treatment]

Huang Z, Jett JH, and Keller RA: Bacteria Genome Fingerprinting by Flow Cytometry. Cytometry 35:169-175, 1999.

Powerful New Tool for Drug Discovery. An interdisciplinary team of investigators, collaborating with researchers at the yeast genome resource at the University of Washington,

Seattle, have developed a novel technique for identifying molecules that are essential to specific cellular activities and may therefore serve as targets for new drug development. The scientists used powerful genetic selection and screening methods to first find small molecules that inhibit specific biological pathways in yeast and then to identify the cellular peptides the inhibitors bound. Because many cellular peptides that are essential to yeast pathways are similarly critical to the operation of human cells, the technique may pinpoint new leads for drug discovery and shed light on the complex biochemical underpinnings of human health and disease. [secondary B treatment]

Norman TC, Smith DL, Sorger PK, Drees BL, O=Rourke SM, Huges TR, Roberts BL, Friend SH, Fields S, and Murray AW: Genetic selection of peptide inhibitors of biological pathways. Science 285:591-595, 1999.

Human Sequencing Quality. The sheer volume of genomic DNA being sequenced by the Human Genome Project is increasing dramatically. As with any complex production effort, it is important to ensure the effort meets appropriate quality standards. The international Human Genome Project has agreed that the sequence should have no more than 1 error in 10,000 base pairs, all finished stretches of DNA be at least 30,000 base pairs, and that the sequence should have no gaps except regions that are intractable to current technology. It is expected that the standard for contiguity will be increased as the project progresses.

To ensure that this standard is being met, samples of data were exchanged between sequencing groups and assessed for quality. The most recent quality assurance (QA) exercise established that all of the publicly funded U.S. centers sequencing the human genome are meeting, and in many cases exceeding, the established standards. In the near future, an independent QA center will be established to continue to monitor the rapidly increasing amount of genomic sequence being deposited in public databases.

http://www.nhgri.nih.gov:80/Grant_info/Funding/Statements/RFA/quality_standard.html

Felsenfeld A, Peterson J, Schloss J, Guyer M: Assessing the quality of the DNA sequence from the Human Genome Project. Genome Research 9:1-4, 1999.

Genetics Resources on the Web (GROW). As an increasing proportion of both health professionals and the public turn to the World Wide Web for information about human genetics, they are confronted with a proliferating number of sites that offer information in this area. Recognizing that this proliferation offers both the promise of improved access to helpful information but also the peril of wasting resources through duplicative effort and even of creating misinformation, the NIH convened a meeting on August 17, 1999 to address AGenetics Resources on the Web (GROW).@ Twenty-eight organizations, including voluntary health organizations, other interested non-profits, genetics services providers and federal agencies attended. The attendees enthusiastically committed to work cooperatively under the auspices of GROW to optimize the provision of human genetics information on the web. They established working groups and, to facilitate ongoing communication, a listserv and decided to meet again at the NIH in March 2000.

Genetics Education Resources. As genetics revolutionizes medicine, our citizenry must have the basic knowledge to make informed decisions about their health. A key target audience for efforts to enhance genetic literacy is high school students. The NIH Office of Science Education has produced a teaching module on human genetic variation to supplement the high school biology curriculums. The module is being distributed to biology teachers nationwide. The curriculum supplement includes a teacher-s guide, background information, activities, student worksheets, and a supplemental CD-ROM. Students will be able to use the CD-ROM to view videos and simulations and to conduct research.

A New Approach to Tissue Engineering: DNA Delivery via Polymer Matrices. Tissue engineering is emerging as an approach for the repair or replacement of body tissues and organs, with multiple technologies under investigation. One promising new technology uses a polymer matrix system to achieve sustained release of plasmid DNA for periods from 10-30 days. In a rat model, the quantity of tissue-inducing proteins secreted as a result of the delivery system was sufficient for tissue formation. Future studies will explore using this gene therapy approach to engineer specific tissues. [secondary B treatment]

Shea LD, Smiley E, Bonadio J, and Mooney DJ: DNA delivery from polymer matrices for tissue engineering. Nature Biotechnology 17: 551-54, 1999.

Improving Farmworker Monitoring Systems. Good intentions can go awry. Such was the case in California where NIH-supported scientists found that an extensive state monitoring program aimed at measuring farmworker exposure to pesticides fell far short of its goal. The routine assays required in farmworkers examined blood levels of acetylcholinesterase, an enzyme that serves as a marker of exposure to organophosphate and carbamate pesticides. What these researchers discovered was that the commercial bioassays being used were inaccurate, often underestimating enzyme levels (and thus exposure effects) by as much as 40%. In effect, the extensive statewide monitoring program could not provide accurate or useful information. As a result of these findings, the California Environmental Protection Agency rewrote its Public Worker Safety Regulations. The regulations now require standardized procedures for clinical laboratories for assessment of acetylcholinesterase activity in agricultural workers exposed to organophosphate and carbamate insecticides. Thus the implementation of the law is now in line with the public health intent of the law.

State of California, Worker Health and Safety Branch. (1999) Pesticide Workder Safety Regulations Health and Safety Report HS-36.

First Chemical Molecular Motor Developed. Miniaturization is a major thrust of modern engineering, including the design and fabrication of integrated circuits, microfluidics, and nanoscale machines. Now, basic researchers have for the first time designed and synthesized a molecular-scale motor that transforms chemical energy into controlled, unidirectional rotary movement. This research offers an atomic-level explanation of the operation of well-known but poorly understood biological motors such as cilia and flagella, and provides a basis for the design of machines that are far smaller than any that have been devised to date.

Kelly TR, De Silva H, and Silva RA: Unidirectional rotary motion in a molecular system. Nature: in press.

Advances in Structural Biology. NIH investigators continue to develop and apply innovative techniques to determine the structures of biologically significant proteins and use these structures to help study protein function. Recent accomplishments include studies on the crystal structure of cyanovirin-N, a potent HIV-inactivating protein; determination of the solution structure of a portion of HIV-integrase, the enzyme responsible for HIV incorporation into human cells, and further elucidation of HIV-integrase function; determination of the solution structure of a key protein complex involved in bacterial phosphate transfer which plays a central role in signaling pathways essential to cellular function; and determination of the crystal structure of a membrane protein-binding domain that regulates cell membrane trafficking and signaling pathways. An understanding of the structure and function of these proteins is essential for the design and development of new drugs that can treat or prevent the disease process. Researchers have also developed and applied novel techniques to the study of large proteins, allowing the characterization of protein dynamics to be extended to larger protein systems. These studies hold promise in improving the accuracy of structures determined and have propelled the field of protein structure determination into that of larger proteins.

Yang F et al., *Crystal Structure of Cyanovirin-N, a Potent HIV-Inactivating Protein, Shows Unexpected Domain Swapping.* J. Mol. Biol. 1999; 288:403-12.

Cai M, Huang Y, Caffrey M, Zheng R, Craigie R, Clore GM, and Gronenborn AM, *Solution structure of the His12 -- Cys mutant of the N-terminal zinc binding domain of HIV-1 integrase complexed to cadmium.* Protein Sci 1998 Dec;7(12):2669-74.

Esposito D and Craigie R, *HIV integrase structure and function*. Adv Virus Res 1999;52:319-33.

Garrett DS, Seok YJ, Peterkofsky A, Gronenborn AM, and Clore GM, *Solution structure of the 40,000 Mr phosphoryl transfer complex between the N-terminal domain of enzyme I and Hpr.* Nat Struct Biol 1999; 6(2):166-73.

Caffrey M, Kaufman J, Stahl SJ, Wingfield PT, Gronenborn AM, and Clore GM, *3D NMR experiments for measuring 15N relaxation data of large proteins: application to the 44 kDa ectodomain of SIV gp41*. J Magn Reson 1998;135(2):368-72 .

Misra S and Hurley JH, *Crystal Structure of a Phosphatidylinositol 3-phosphate-specific membrane targeting motif, the FYVE domain of Vps27p.* Cell 1999; 97:657-66.

Life Without Fat. White adipose tissue (WAT) is the major organ for regulated storage of triglycerides for use as metabolic energy. WAT helps control energy balance, including food intake, metabolic efficiency, and energy expenditure via its secreted hormone, leptin, and possibly other unidentified hormones. Leptin was discovered as the product of the obesity gene in animals, and plays a prominent role in humans as well, regulating energy metabolism by sending signals within the brain and between the brain and the body. Excess body fat, or obesity, is a major health problem in the United States, increasing the risk of diabetes, high blood pressure, and heart disease. However, the mechanisms by which obesity causes these diseases are unclear. To help understand the role of fat tissue in diabetes and metabolism, NIH researchers have generated a transgenic mouse with essentially no WAT and examined the contribution of WAT to energy metabolism, reproductive function and disease susceptibility. They found that these mice have poor fertility; enlarged organs; elevated levels of glucose, insulin, free fatty acids, and triglycerides; low levels of leptin; and early death, indicating that fat tissue has some beneficial effects. Manifestations in the mouse model were similar to those seen in human lipoatrophic diabetes. Lipoatrophic diabetes is a consequence of the absence of fat, suggesting that a lack of fat is causative for the human disease. Lipoatrophy is paradoxical, with the lack of fat causing diabetes. The usual scenario is that obesity causes type 2 diabetes. Therefore, it is essential to study similarities between these two disorders.

Life Without Fat: A Transgenic Mouse. Moitra J et al. Genes and Development 1999; 12(20):3168-3181

Role of T Cells In Hepatitis C. In individuals infected with the hepatitis C virus (HCV), it is believed that T cell responsesBcritical cells involved in the immune responseBare critical in determining HCV persistence and disease outcome. Identifying the mechanisms for HCV persistence is one of the biggest challenges faced by scientists. Recently, a novel method to identify specific T cells activated during an immune response has been developed. NIH investigators have used this model to identify HCV-specific cytotoxic T lymphocytes (CTLs), T cells which play an important role in the development of liver injury as well as clearance of the virus, and found these cells in the blood and liver of HCV-infected patients. Surface characteristics (phenotypes) of these T cells varied in different patients, suggesting that HCV may elicit very different immune responses in different individuals. They also found that the frequency of HCV-specific CTLs in the liver was higher than in blood. Direct quantitation and characterization of these cells using this novel method should extend our understanding of the immune reactions in the development of the disease and the mechanism of clearance and persistence of HCV.

Quantitative Analysis of Hepatitis C Virus-Specific CD8+ T Cells in Peripheral Blood and Liver Using Peptide-MHC Tetramers. He XS et al. PNAS 1999; 96(10):5692-5697.

Cn3D 2.5 for Structural Analysis. The relationship between a molecule's structure and its function is at the root of both normal and abnormal biochemical processes. Since molecules that share similar underlying amino acid or DNA sequences often share similar structures as well, an important area of research focuses on methods of accurately predicting a molecule's structure

based on its underlying sequence. The National Center for Biotechnology Information's latest release of Cn3D, a powerful software package for viewing and comparing the three-dimensional structure of molecules, provides an integrated approach to combined analysis of sequence and structural similarities. This new analytical capability arms researchers with a versatile tool for taking a molecular sequence for which structural and functional information is lacking, mapping that to a model structure, and thereby gaining insight into its potential function in living cells.

Cn3D Web site: http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.html

PHI-BLAST: Motif-Constrained Sequence Similarity Searches. Comparison, whether of morphological features or protein sequences, lies at the heart of biology. Similar sequences are often found to have similar function. The BLAST (Basic Local Alignment Search Tool) family of programs developed by the National Center for Biotechnology Information has made it easier to rapidly scan huge sequence databases for overt similarities, and its widespread impact is reflected in the fact that the original paper describing the method is the most heavily cited scientific paper of the 1990's. However, not all significant homologies are overt; some of the most interesting are subtle and may not be detected with a standard BLAST search. The PHI-BLAST program extends the power of BLAST to detect weak, but significant, relationships by incorporating motif-based hypotheses as to biological function directly into the analysis. As a new tool for cutting through the huge amount of available protein sequence data, PHI-BLAST has great potential to discover the function of genes that have been sequenced, but for which function is still unknown.

Zhang, Z, AA Schäffer, W Miller, TL Madden, DJ Lipman, EV Koonin, and SF Altschul. Protein sequence similarity searches using patterns as seeds. *Nucleic Acids Res* 26(17):3986_3990, 1998.

SAGEmap: Measuring Gene Expression. Serial Analysis of Gene Expression, or SAGE, is an experimental technique designed to quantitatively measure gene expression in cells. The SAGE technique itself includes several steps utilizing molecular biological, DNA sequencing and bioinformatics techniques, and produces short DNA sequence "tags" that can be associated with specific genes. A major application of SAGE is in the identification of abnormal gene expression leading to, or diagnostic of, various disease states, such as cancer. The SAGEmap Web service, introduced by the National Center for Biotechnology Information in March 1999, implements many functions useful in the analysis of SAGE data, including that generated for the Cancer Genome Anatomy Project. It is hoped that SAGE information can be used to gain insight into the initiation and development of cancer in the human body. SAGEmap provides the tools that researchers need to utilize gene expression data to quickly determine, at the molecular level, the causes of disease.

SAGEmap Web site: http://www.ncbi.nlm.nih.gov/SAGE/

Human Genome Resources. The NIH National Center for Biotechnology Information offers a Human Genome Resources Web service, which supports the Human Genome Project by providing integrated and centralized to a full range of human genome data available from NCBI and external sources. In addition to core research databases such as Unigene and GeneMap99, which are key tools for gene discovery, this central service provides links to sites of more general interest, such as Genes and Disease, which gives descriptive synopses of more than 60 diseases of genetic origin. The Human Genome Sequencing component tracks the progress on the complete sequencing of the human genome and provides access to growing amount of contiguous sequence data for each chromosome. The full range of Human Genome Resources offered by the National Center for Biotechnology Information provides an essential framework for the exploration of the human genome and will accelerate the identification of disease-associated genes.

Human Genome Resources Web site: http://www.ncbi.nlm.nih.gov/genome/guide/

AT-hook Found in Many Chromosomal/DNA-Binding Proteins. The AT-hook is a protein motif first recognized in the high mobility group non-histone chromosomal binding protein HMG-I(Y). Researchers from the National Center for Biotechnology Information have scanned the non-redundant protein sequence database using sensitive search tools to find all instances of this motif. The results of this survey indicate that the AT-hook motif is specific to known or predicted chromosomal/DNA-binding proteins, implying a possible function as a general DNA minor groove binding module. Many physiological processes are regulated via the binding of proteins to DNA, and the discovery of this DNA-binding motif in chromosomal/DNA binding proteins will increase our understanding of these mechanisms.

Aravind, L, and D Landsman. AT-hook motifs identified in a wide variety of DNA-binding proteins. *Nucleic Acids Res* 26(19):4413-21, 1998.

DNA Replication May Have Evolved Twice. Although the components of transcription machinery are highly conserved in the bacteria and the archaea/eukaryota, several components of the DNA replication apparatus appear to be either unrelated or only distantly related between the two groups. Researchers at the National Center for Biotechnology Information conducted a detailed comparison of the proteins essential to DNA replication in the bacteria and archaei/eukaryote. This comparison confirmed that key DNA replication proteins differ between these two lineages, prompting the interesting suggestion that the last common ancestor of bacteria and the archaea/eukaryote used a genetic system comprised of both DNA *and* RNA, and that modern DNA replication subsequently evolved independently in the two lineages. Since DNA replication systems are often the target of therapeutic agents, a knowledge of significant differences in the DNA replication systems of eukaryotes, such as man, and pathogens, such as bacteria, will be valuable in the development of new drug therapies.

Leipe, DD, L Aravind, and EV Koonin. Did DNA replication evolve twice independently? *Nucleic Acids Res* 27(17):3389_3401, 1999.

KARIBIN: Karyotypic Region-Based Integration of Chromosomal Information. KARIBIN is a Web-based service that provides a comprehensive view of the integrated mapping and sequencing data for the human genome. This information resource links cytogenetic chromosomal bands to a wide range of data, including genetic and physical maps, GeneMap99, YAC and BAC/PAC clones, disease phenotypes and high throughput genomic sequences generated through the Human Genome Sequencing Project. By providing a framework for the integration of many types of chromosomal information, KARIBIN has great potential to accelerate the process of disease gene hunting.

Zhang, J, G Shen-Ong, and J Ostell. AKARABIN,@ an information resource for obtaining genomic information in a cytogenetic band. *Genome Res* 9(1):91_8, 1999.

Multilateral Initiative on Malaria. NIH has led an international effort to provide malaria researchers in Africa with full access to the Internet and the resources of the World Wide Web. This project began with NIH's leadership in the Multilateral Initiative on Malaria in which African scientists identified electronic communication and access to scientific information as critical in the fight against the devastating and economically debilitating effects of malaria in developing countries. Results at the completion of the Alpha Phase and Phase 1: researchers at the Malaria Research and Training Center in Bamako, Mali, are connected by radio waves to their local Internet Service Provider and their colleagues at the CDC/KEMRI and Wellcome/KEMRI sites in Kenya now fully connected via satellite for data, voice, and image. Phase 2 will comprise two sites in Ghana which engaged in vaccine testing. Partners for the initial phases, in addition to the NIH, included: Centers for Disease Control, Wellcome Trust, World Bank.

http://www.mimcom.net

To be published: Royall, Julia, Elliot Siegel, and Mark Bennett, "Wires, Webs, and MIMCom.Net," African Journal of Medicine.

STORIES OF DISCOVERY

Artificial Skin Offers Hope for Burn Victims

A 3-year-old girl grabs a frying pan of boiling-hot oil off the stove . . . while playing with matches, a 5-year-old boy ignites his pajamas . . . the tip of an 80-year-old woman's housecoat catches on fire as she reaches for a teakettle on the stove . . . .

Each year in the United States, more than 2 million burn injuries resulting from situations such as these demand medical attention. As many as 10,000 people die every year of burn-related infections, and tragically, many of the victims are children. The good news is that, in recent years, survival statistics for serious burns have improved dramatically. Twenty years ago, second- and third-degree burns covering half the body were routinely fatal. Today, patients with severe burns encompassing 90 percent of their body surface typically survive.

Driving burn injury survival statistics upward have been fundamental advances in basic research aimed at understanding how skin and the rest of the body respond to damage caused by burns.

Skin Loss Can Deal Lethal Blow

More than simply a protective covering, skin is a highly dynamic network of cells, nerves, and blood vessels that serves the body in diverse ways. Skin plays an important role in preserving fluid balance and in regulating body temperature and sensation. Immune cells resident in skin help the body prevent and fight disease. Burn-induced skin loss affords bacteria and other microorganisms easy access to the warm, moist, nutrient-rich fluids that course through the body, while at the same time it provides a conduit for the rapid and dangerous loss of these fluids. Extensive fluid loss can thrust a burn or trauma victim into shock, a life-threatening condition in which blood pressure plunges so low that vital organsBsuch as the brain, heart, and kidneysBsimply cannot get enough blood (and thereby oxygen) to function.

Replenishing skin lost to severe burns is an urgent matter in the care of a burn patient. In the case of burns covering a significant portion of the body, two immediate tasks come to the fore. First, the burned skin must be stripped, then the unprotected underlying tissue must be quickly covered. Antibiotic treatment buys some time by limiting potentially deadly infections. Despite being seemingly obvious, these important steps in the immediate care of a burn patient are the result of decades of carefully conducted research on how the body responds to burn injury.

In the early 1970s, a group of burn injury researchers reviewing the grim mortality statistics facing burn patients at the time reasoned that the complete removal of badly burned skin (as opposed to letting it slough off over time) might offer greater protection against wound infection and improve the very poor prognosis that these patients faced. Recognizing that a necessary follow-up would be immediate and permanent skin replacement, these scientists pioneered the use of skin from related donors (such as family members with similar genetic markers). But doing so required that the burn patient be given powerful immunosuppressant drugs to dampen

the immune system and prevent rejection of the graft. Unfortunately, crippling the patient's immune system in this way posed many serious problems. The need for some form of "artificial skin" became urgently apparent.

Soon thereafter, with support from the National Institutes of Health, the researchers developed the first version of an artificial skin system called Integra J. Every similar artificial skin product that has since been researched and developed hinges upon the conceptual framework that eventually yielded this product. Today, Integra J is used to treat 1 of every 10 severely burned patients in the United States and is the top-selling skin substitute in the world.

Artificial Skin: Born of a Marriage Between Engineering and Medicine

The brainchild of a trauma surgeon and a mechanical engineer, Integra J is a prime example of the extraordinary value of collaborative research.

Integra J contains no living components and is not actually designed to be a replacement skin. Rather, it supplies a protective covering and a pliable scaffold onto which the patient's own skin cells can "regenerate" the lower, dermal layer of skin destroyed by a severe burn. Integra J consists of two layers, just as living skin is structured. The bottom, dermal-like layer is composed of a matrix of interwoven bovine collagen (a fibrous cow protein) and a sticky carbohydrate (sugar) molecule called glycosaminoglycan that mimics the fibrous pattern of dermis. This matrix is then affixed to a removable upper layer: a medical-grade, flexible silicon sheet that mimics the epidermal, or surface, layer of skin. The product looks somewhat like translucent plastic wrap.

After first removing tissue destroyed by the burn, a burn surgeon drapes Integra J over the wounded area of the patient and leaves it there for 2 to 4 weeks, during which time the patient's own cells make their way into the matrix and create a new dermis. The top layer of Integra J is then removed, and a very thin sheet of the patient's own epithelial cells is applied. Over time, a normal epidermis (except for the absence of hair follicles) is reconstructed from these cells.

On the Horizon

Integra J moved from the research lab to licensing, testing, and production by Marion Laboratories of Kansas City, Missouri. The product is now being manufactured and sold by Integra LifeSciences Corporation of Plainsboro, New Jersey. After extensive clinical testing, Integra J received FDA approval in the mid-1990s, and is now enjoying wide use for the treatment of severe burns and other serious skin injuries. Through an NIH Small Business Innovation Research grant, Integra LifeSciences is currently investigating what potential benefits adding a molecule that coaxes new growth of blood vessels within the original matrix might add to the quality and/or speed of skin regeneration of a burn-wounded area. Early results are promising, showing that this approach can speed healing and improve the physical appearance of the once-burned area.

Other scientists have succeeded in expanding a small number of real skin cells into a transplantable sheet that can be layered on top of Integra J that has been bathed in a nutritious mix of growth factors. The method has been evaluated in a small number of patients, and so far appears to offer a significant advantage over other currently available technologies.

Turning Blue Babies Pink

In 1944, Eileen Saxon, a blue, frail 15-month-old child weighing little more than a newborn, was anesthetized with drops of ether and woke up a pink pioneer in congenital heart disease. She had the first Ablue baby@ operation conceived and perfected by the team of Alfred Blalock, Helen Taussig, and Vivien Thomas at Johns Hopkins, which revolutionized congenital heart disease treatment. Her postoperative course was rocky. The sophisticated monitoring commonplace in pediatric hospitals today was nowhere in evidence. Instead, the surgical team visited frequently, and a pediatrician set up a stretcher next to Eileen=s bed, remaining by the child=s side continuously for the first 48 hours.

Eileen=s malformation, tetralogy of Fallot, is the most common cause of cyanotic (blue) congenital heart disease. Pathologists began describing the constellation of features from autopsy specimens in the late 1600s, and Fallot, writing in 1888, summarized the four consistent features: a large hole between the 2 pumping chambers of the heart, an underdeveloped blood supply to the lungs, an abnormally positioned aorta, and thickening of the right ventricle. For over 250 years, physicians could only stand by and watch while children with cyanotic heart conditions suffered through childhood, rarely surviving into adolescence. The Blalock-Taussig shunt became the medical equivalent of a shot heard =round the world.

Amazingly, Eileen was taken to the operating room for heart surgery without any direct imaging of her heart to pinpoint the diagnosis. In 1944, doctors had at their disposal only primitive electrocardiography, chest X-rays, and fluoroscopy to augment patient history and physical examination in making a cardiac diagnosis. From these tools, inferences could be made about the shape of the heart and the size of the ventricles, but direct confirmation came only at autopsy. The success of the blue baby operation in providing the first therapy for congenital heart disease led to an explosion of interest in better diagnostic tools. Fortuitously, this coincided with the establishment of the National Heart Institute within the NIH (now the National Heart, Lung, and Blood InstituteBNHLBI) in 1948. From its beginning, the Institute supported research in the new field of pediatric cardiology. In 1950, it awarded Dr. Blalock $12,000 to study surgical approaches to congenital heart disease. His work built on a previous award to Johns Hopkins to study radiographic and angiocardiographic diagnosis of congenital heart disease. Researchers at Hopkins assembled a primitive angiographic apparatus that would advance film cassettes using a rope pulley for serial imaging during the dye injection. More often than not, one of them would have to remain under the contraption, guiding the cassettes so they did not fall on the floor, and simultaneously trying to avoid X-ray beams. From this humble beginning arose modern angiocardiography, which meant that, for the first time, anatomy inside the heart could be visualized in the living child outside the operating room.

Whereas the Baltimore team sought to create a shunt to the pulmonary artery, Dr. Robert Gross was the first surgeon to eliminate a naturally occurring shunt, the ductus arteriosus. This vessel allows blood to bypass the lungs in the fetus, and is programmed to close shortly after birth. Occasionally, however, it does not close, and can overload the left side of the heart, leading to heart failure. In 1938, Dr. Gross was a surgical resident at the Children=s Hospital in Boston. He approached the Chief of Surgery with a proposal to tie off the ductus in a child in whom the

diagnosis had been made by auscultation (listening with a stethoscope). The Chief rejected Gross= proposal in no uncertain terms, but persistence paid off. While the Chief was on vacation, Gross successfully ligated the ductus in a 7-year-old child who survived and did well.

The next major breakthrough was the development of heart-lung bypass, which allowed surgery to be performed on the inside of the heart in a bloodless field. Dr. John Gibbon, a surgeon at the Massachusetts General Hospital, began work on a heart-lung bypass machine in the 1930s. His work was interrupted by World War II, but he resumed it after the war with support from the NIH and collaboration with IBM engineers. His first patient was a 15-month-old baby who died, in part, because her preoperative diagnosis was incorrect. Success came with his second patient, an 18-year-old girl with a hole between the two top chambers of her heart (atrial septal defect), who survived the first heart-lung bypass procedure in 1953. Although many refinements were needed to bring bypass into widespread use, it was a big improvement over previous practices of packing the patient in ice and doing Abeat-the-clock@ surgery, or of so-called cross-circulation, in which the blood supply of a parent and the child were connected, and the parent=s heart provided the circulating pump for the child. This latter procedure has been described as the only surgical procedure with the potential for 200 percent mortality, and it was quickly replaced.

Through courage and resourcefulness on the part of patients and physicians, it had now been demonstrated that both open- and closed-heart surgery were feasible on infants and children. Angiography provided general diagnosis of congenital heart disease, but it was invasive, requiring catheters to be placed through peripheral blood vessels into the heart. With successful surgery becoming more widespread, there was increased interest in developing noninvasive imaging. In 1957, the NIH awarded a grant to Alexander Nadas at Boston Children=s Hospital to study phonocardiography in congenital heart disease. A microphone was placed on the child=s chest so that the sounds made by murmurs and by heart valves opening and closing could be recorded on paper for further study. From these tracings, physicians quantitated the degree of narrowing of heart valves and recognized additional heart sounds that indicated heart disease.

The use of ultrasound waves to visualize the heart (echocardiography) was an astute clinical application of sonar technology developed during World War II. Fuzzy images barely recognizable as the heart were produced for the first time in 1954. The scientific details of echocardiography were worked out largely by physicists and engineers, including Dr. Olaf Von Ramm, a biomedical engineer at Duke University. Dr Harvey Feigenbaum, a cardiologist at the Indiana University School of Medicine was the first to realize the practical potential and to bring echocardiography into clinical practice. Both researchers, as well as many other adult and pediatric cardiologists working on echocardiography, were supported by the NIH and continue to receive NIH funding. Echocardiography was first used in children in the early 1970s. By the 1980s, ultrasound techniques had been refined, color imaging had been added, and image resolution had improved to the point that noninvasive diagnosis of congenital heart defects could be reliably performed in utero. Dr. von Ramm now is applying his energies and NIH funds to develop 3-D echocardiographic imaging, first introduced in 1995. Commercial 3-D systems, now in experimental use in both adults and children, allow researchers to peer inside of hearts in ways that are not possible with conventional 2-D imaging.

Once hearts could be imaged, and congenital heart disease could be diagnosed accurately, epidemiologic studies could be done to determine the patterns of congenital heart defects in populations. The landmark Baltimore-Washington Infant Study, funded by the NIH in the 1980s, is the gold standard for categorizing types of congenital heart disease, estimating their occurrence among live-born infants, and analyzing possible risk factors. From this study we learned that about 1 percent of newborns (about 40,000 per year in the United States) have some form of congenital heart disease, making this the most common birth defect.

A child born 50 years ago with a heart defect had a dismal prognosis. Today, that same child will likely live a long and productive life. Looking back over the past half century, one cannot help but be awestruck by the incomparable progress in pediatric cardiology. In no other pediatric specialty has the medical landscape changed so dramatically, from near-certain mortality at an early age, to prenatal diagnosis and, in some cases, prenatal therapy. Delicate repair of complex congenital heart defects can now be undertaken in the newborn period, on infants weighing as little as 3 pounds. These accomplishments are founded on the courage of families willing to submit their desperately ill children to unproven procedures to forestall death, and to the intellect, persistence, and skill of physicians and researchers who pioneered innovative therapies. The NIH has been a constant partner in the research that supported every step of this miraculous journey, from the development of echocardiography, angiocardiography, and surgical procedures in children to the now common clinical use of genetic testing for abnormalities associated with congenital heart disease. Looking forward to the next 50 years, the NIH has taken the leadership role in supporting research into the molecular underpinnings of normal and abnormal heart development. With new molecular and physiologic tools, understanding the reasons why heart development goes awry may lead to therapies unimagined today.

The Visible Humans

The Visible Human Project began as a result of a long-range planning effort some thirteen years ago. It foresaw a coming era where bibliographic and factual database services would be complemented by libraries of digital images, distributed over high speed computer networks and by high capacity physical media. The plan foresaw the increasing role of electronically represented images in clinical medicine and biomedical research. Early in 1989, an ad hoc planning panel was convened and made the following recommendation: "NLM should undertake a first project building a digital image library of volumetric data representing a complete, normal adult male and female. This Visible Human Project will include digitized photographic images for cryosectioning, digital images derived from computerized tomography and digital magnetic resonance images of cadavers."

Beginning in 1991, the Visible Human Project acquired transverse CT, MRI and cryosection images of a representative male and female cadaver. The Visible Human Male data set consists of MRI, CT and anatomical images. Axial MRI images of the head and neck and longitudinal sections of the rest of the body were obtained at 4 mm intervals. The MRI images are 256 pixel by 256 pixel resolution. Each pixel has 12 bits of grey tone resolution. The CT data consists of axial CT scans of the entire body taken at 1 mm intervals at a resolution of 512 pixels by 512 pixels where each pixel is made up of 12 bits of grey tone. The axial anatomical images are 2048 pixels by 1216 pixels where each pixel is defined by 24 bits of color, about 7.5 megabytes. The anatomical cross-sections are also at 1 mm intervals and coincide with the CT axial images. There are 1871 cross-sections for each mode, CT and anatomy. The complete male data set is 15 gigabytes in size.

The Visible Human Female data set has the same characteristics as the male cadaver with one exception. The axial anatomical images were obtained at 0.33 mm intervals instead of 1.0 mm intervals. This resulted in over 5,000 anatomical images. The data set is about 40 gigabytes. The spacing in the "Z" direction was reduced to 0.33 mm in order to match the 0.33mm pixel spacing in the "XY" plane. This enables researchers who are interested in three-dimensional reconstructions to work with cubic voxels.

Now that the data collection phase of the Visible Human Project is completed, a second phase has begunBthe segmentation, classification, and three-dimensional rendering of the data set. A new research effort is under way. Its ultimate objective is to identify all anatomical structures within the Visible Human data set including the extent of each structure. Work has begun on the male thorax. Each object in each cross-section will be labeled, and the relationship of each object to the other objects in its cross-section and in the adjacent cross-sections will be catalogued. The extent of a single object that spans several cross-sections will be noted. I n order to accomplish this, information about building geographic databases and databases associated with computer-aided drafting systems will be used as starting points for developing an interactive anatomical digital atlas.

Two more projects are underway. The first will result in a complete atlas of the human head and neck regions that will form the prototype for a new wave of educational applications based on the

Visible Human Project data set coupled with other human imagery sources and interactive media. The second will create a self-sustaining development effort to support image analysis research in segmentation, classification and deformable registration of medical images.

The Visible Human Project data sets are designed to serve as a common reference point for the study of human anatomy, as a set of common public domain data for testing medical imaging algorithms, and as a test bed and model for the construction of image libraries that can be accessed through networks. The data sets are being applied to a wide range of educational, diagnostic, treatment planning, virtual reality, artistic, mathematical and industrial uses by over 1,200 licensees in 41 countries. Some of these applications include surgical simulators, non-invasive colon cancer screening, simplified plastic surgery, prostate cancer surgical rehearsal, revolutionizing the study of anatomy in high school, college, and professional school, radiation absorption modeling, crash testing, and artistic renderings and imaging. But key issues remain in the development of methods to link such image data to text-based data. Standards do not currently exist for such linkages. Basic research is needed in the description and representation of image based structures, and to connect image-based structural-anatomical data to text-based functional-physiological data. This is the larger, long-term goal of the Visible Human Project: to link the print library of functional-physiological knowledge with the image library of structural-anatomical knowledge into one unified resource of health information.

MEDLINE: A Continuing Story of Discovery

Medical practitioners and medical researchers both depend on the accumulated wisdom of those who have gone before. It was not so long ago that one could assemble this wisdom only by poring over printed bibliographies, usually the *Index Medicus*, which was published beginning in 1879. Today, virtually all biomedical scientists, many health practitioners, and an increasing number of consumers use a variety of methods to search the MEDLINE database to learn about published research findings. This is the story of how an NIH Adiscovery@ has evolved over the decades to become a service that, in the words of Vice President Al Gore, Amay do more to reform and improve the quality of health care in the United States than anything else we have done in a long time.@

The pioneering MEDLINE project, begun in the early seventies, evolved from the computerized system used to produce the *Index Medicus*, which had been installed in 1964. MEDLINE was the first successful marriage of a large reference database with a national telecommunications network, and it has been called the Model T of online databases: although it would usually get you where you wanted to go, it required a pioneering spirit to master its intricacies and the patience of Job to deal with its idiosyncrasies. Even so, more requests were received than could handled from medical librarians who wanted to be trained so that they could provide literature search services for health professionals and scientists in hospitals, universities, and laboratories. The original system covered 239 journals and it was boasted that it was Acapable of supporting up to 25 simultaneous users.@

A typical story from the seventies: Unnecessary and dangerous surgery is avoided when a hospital librarian in New Jersey, called on to do a MEDLINE search about a rare case of thalassemia with spinal cord compression, retrieves one pertinent reference that has the information needed to guide the physician.

The eighties saw the introduction of AGrateful Med,@ a software program created that one could load onto a PC or Macintosh and, equipped with a modem and a password, search MEDLINE right from one=s home, office, or laboratory. Grateful Med was eagerly snapped up not only by librarians but by health professionals, scientists, students, lawyers, medical journalists and others, who saw the average charge of $2 per MEDLINE search as a bargain. Trying to find the same information in the printed *Index Medicus* would surely cost much more, in time and effort, if it could be done at all. At the height of the Grateful Med era, the number of registered users reached 125,000 and coverage had increased to almost 4,000 journals. Another change by then was that most MEDLINE references were accompanied by an abstract.

Typical of Grateful Med users was the Atlanta physician who typed into MEDLINE a boy=s primary complaint of a Anumb chin@ and was able to diagnose from the information he found there that the boy had a form of lymphoma. [If this case sounds vaguely familiar to the reader, it formed part of the story line on an episode of AER.@]

The nineties, of course, is the era of the Internet and the World Wide Web. MEDLINE searching via AInternet Grateful Med@ was introduced in 1996 by Dr. Michael DeBakey, then a Regent of

the Library, and Senator Bill Frist of Tennessee, a surgeon, at a press conference. The following year, Vice President Gore (quoted above) introduced *free* MEDLINE searching via the Web, using a new system called PubMed. Now, for the first time, anyone with access to the Web could search through an immense database of references and abstracts to more than 10 million medical journal articles.

A 1999 experience: AI logged onto the National Library of Medicine=s Web site. A list of 20 articles on ehrlichiosis popped up. One described a new method of testingY.@ The Midwest pathologist called the author at Johns Hopkins, mailed him a blood sample from the patient, and within two days confirmed the tick-borne disease. AWe had nailed it.@

In fact, PubMed and Internet Grateful Med had both simplified MEDLINE searching to the point where the public encountered no difficulty at all in retrieving relevant references on any biomedical subject from the literature. Expecting the average consumer to *understand* much of what was retrieved was another matter. Since about 30 percent of the estimated 180 *million* annual MEDLINE searches were being done by consumers, this represented a virtual epidemic of blank stares and glazed eyeballs. It also presented a wonderful opportunity. Why not create a service that not only will provide selective MEDLINE results that are useful to the consumer, but also link the Web user to the authoritative, full-text health information being put out by NIH institutes and by a variety of non-Federal sources? S uch a service, called MEDLINE*plus*, was introduced in October 1998.

At the same time MEDLINE*plus* was introduced, it was announced that NIH was working with 39 public library systems (some 200 individual libraries) in 9 states and the District of Columbia to equip them to deal with requests for health information from their clientele. MEDLINE*plus* naturally figures prominently in this project. Medical librarians from the National Network of Libraries of Medicine volunteered to work with the public librarians and teach them about MEDLINE and other electronic sources of information. The Board of Regents approved a change in policy to allow the Library to serve the public as well has scientists and health professionals. The program to promote good health information for consumers will receive a boost when, early in FY 2000, a series of Aoutreach awards@ will be made to medical libraries across the nation to enable them to work with local, state, and regional organizations to promote public access.

To sum up, in just the last few years there has been a remarkable change in how consumers seek health information for their personal use. NIH is riding the crest of this wave and is adapting its extremely popular electronic database, MEDLINE, to serve the public as well as it has served scientists and health professionals for three decades.

Neuroprosthetic Devices

The Food and Drug Administration (FDA) has approved a device that, when surgically implanted, can enable some people who are quadriplegic to grasp, hold, and release objects. The Freehand System, made by NeuroControl Corp. of Cleveland, Ohio is the first marketed neural prosthetic to restore function to a paralyzed limb. Now, new experiments demonstrate that animals can be trained to control a robot arm just by thinking about it, which gives some inkling of what the future may hold for restoring lost nervous system function using neuroprosthetic devices.

For centuries, going back to early scientific pioneers like Volta and Galvanni, increased understanding of electricity and the development of tools with which to harness and measure it have been applied to study the nervous system. These efforts are the roots of today=s research in neuroprostheticsBdevices that interface with the nervous system to restore lost sensation or movement.

Since the 1970=s the NIH has been a leader in the support of research that developed neuroprosthetics such as the cochlear implant for the hearing-impaired and a device to provide bladder control for some people with spinal cord injuries. The Freehand System enables people who are quadriplegic, but have some remaining control of the elbow or shoulder, to open and close a hand well enough to hold a pen or hairbrush, pour coffee, and feed themselves. Patients who tested the hand-grasp device reported meaningful improvement in daily activities and greater independence. More than 50,000 people in the United States have spinal injuries for which this device might provide benefit.

The Freehand System consists of a pacemaker-size implant that is placed in the chest wall and connected to electrodes threaded by wire under the skin, down the arm to the forearm and hand muscles. A body mounted joy stick-like control responds to movements of the patient=s shoulder. The shoulder motion sends an electronic signal that the implant electronics translate to commands for muscles of the thumb and finger to pinch together and grasp. With the Freehand System, patients require training and physical rehabilitation to learn to use the device effectively, and, as with any implanted device, there may be swelling and skin irritation, and technical problems that require adjustment, but many people who have tried the system believe the benefits are well worth the trouble.

The principle behind a neural prosthesis such as the Freehand System is simple: Apply electrical stimulation to activate a nerve or muscle to restore movement. In fact, observations of a link between electricity and the contraction of muscles date back over two hundred years and include work by the early pioneers in the study of electricity. In practice, however, going from the simple idea to practical neuroprosthetic devices required progress in many areas of science and engineering and decades of applied research by chemists, material scientists, biomedical engineers, physiologists, computer scientists, neurosurgeons, and other experts and professionals. Some of the hurdles that had to be overcomeBand still require further refinementsBinclude:

- $ Hardware: The implanted wires, electrodes and other components must be small and compatible with the human body both so the devices do not cause harm to the person and so the body=s tissues and fluids do not cause the device to malfunction.

- $ Electrical Stimulation: The amount and pattern of electrical stimulation delivered to the nerves and muscles must be carefully determined to elicit the desired activity and yet not cause injury.

- $ Nervous System: The anatomy of the nervous system must be mapped so that electrodes can be placed precisely to stimulate the correct points and the physiology must be understood so the device can be designed to provide appropriate signals to muscle.

- $ Cost/Benefit ratio: The complete system must be reliable, compact, and easy to use so that the benefits outweigh any burden to the user in terms of cost, time involved in learning to use the system, technical adjustments, and side effects.

What has been accomplished so far in this pioneering field is just the beginning of what may be achievable to restore muscular control, hearing, vision, and touch. Recent experiments in rats give some inkling of what the future may hold. By wiring directly into the brain, scientists have trained rats to control a robot arm just by thinking about it. First rats were trained in a conventional way to press a lever with their paws to move a robot arm. During this behavior the investigators recorded the activity of several dozen nerve cells through arrays of electrodes implanted in the rats= brains. A sophisticated analysis of the nerve cells= activity revealed a group of cells whose activity predicted the movement of the robot arm. When the signals from these 32 cells were amplified, combined appropriately, and sent directly to the robot arm controller, the rats quickly learned to directly control the arm just by their brains= activity, without using their paws.

This work, as well as other research to develop neuroprosthetics, has involved scientists, physicians, and engineers from many disciplines, collaboration and support from other NIH components, government agencies, and voluntary organizations, as well as interaction with the private sector. The efforts have been stimulated and coordinated, in part, by an annual meeting sponsored by the NIH Neural Prosthesis Program to bring together people involved in all aspects of neuroprosthetics.

Research is continuing to develop the technology that will assist people with spinal cord injury, stroke, or head injury to stand and will assist the visually-impaired to see. These efforts, combined with other medical research to develop strategies to prevent or lessen the amount of nervous system damage following injury or disease or to regenerate lost nerve cells, offer hope to the many thousands of people who become physically disabled each year because of impaired nervous system function.

Synchrotrons Illuminate Atomic Architecture of Life

The principle "form follows function" is espoused by designers and architects who believe that the shapes of objects should suit their intended use. This principle also holds true in biology. In fact, form is so critical to the function of biological molecules that even the slightest alteration in a protein's three-dimensional (3-D) structure can produce a life-threatening disorder such as sickle cell anemia, Lou Gehrig=s disease, or an abnormal heartbeat. By studying the minute details of molecular shape and its impact on the human body, structural biologists have gained a deeper understanding of the molecular bases of disease and have used this knowledge to devise improved therapies for diseases ranging from AIDS to the common cold. Many of these discoveries depended on the use of a very bright and versatile type of light known as synchrotron radiation.

Synchrotron radiation is produced when electrons or other subatomic particles are forced to accelerate inside a huge circular chamber, usually ranging from 200 feet to about 1 mile in circumference. As the particles accelerate around the path, they radiate a wide spectrum of light, including intense and brilliant X rays. When physicists first observed synchrotron radiation in the late 1940s, they considered it more of a nuisance than an asset; the massive synchrotron facilities were originally built for studies of subatomic particles, and the radiation was regarded as an unwanted energy leak. But by the early 1970s, a handful of researchers had recognized that this intense type of light could be harnessed to probe the structures of matterBincluding biological moleculesBby x-ray techniques, and they rallied to build laboratories adjacent to the synchrotron rings to tap this otherwise-wasted resource.

X-ray diffraction had been used since the 1950s to uncover the structures of dozens of biologically important compounds at near-atomic resolution, including hemoglobin, myoglobin, vitamin B12, and DNA. By shining X rays through crystallized molecules from many different angles and then capturing the patterns produced by diffracted X rays, biologists were able to calculate how the X rays had been deflected, and from this mathematically deduce the 3-D, atomic structures of the crystallized molecules. However, each structure took several yearsBor even decadesBto solve.

Synchrotron radiation, biologists quickly discovered, offered many advantages over X rays produced by conventional laboratory devices. Because synchrotron radiation is at least 1,000 times brighter, vast quantities of data could be collected from smaller crystals more rapidly. And because synchrotron radiation is also tunable, researchers could select the specific wavelengths for their studies. Despite improvements, x-ray crystallography remained a tedious and frustrating procedure, in part because available instruments and techniques lacked the desired sensitivity.

NIH provided a critical boost to the emerging field in 1980 when it funded one of the nation's first synchrotron resources dedicated solely to biomedical research at the Stanford Synchrotron Radiation Biotechnology Resource. This facility pioneered a new approach to determine directly the phases in protein x-ray diffraction patterns (now usually called MAD phasing). When later combined with techniques of genetic engineering, a powerful new tool for structural biology was created.

Physicists and materials scientists are still the largest user group at U.S. synchrotrons, but the number of structural biologist users has grown dramatically over the past decade, approaching about one-third of the total utilization in 1998. The core operation of four national facilities is funded by the U.S. Department of Energy, and another site at Cornell is funded by the National Science Foundation. In addition to the Stanford facility, NIH now supports beamlines at the following synchrotron radiation facilities: the BioCARS Synchrotron Structural Biology Resource and the Biophysics Collaborative Access Team (BioCAT) in Chicago; the Regional Center for Time-Resolved Synchrotron Spectroscopy and the Macromolecular Crystallography Resource of the National Synchrotron Light Source, both located at Brookhaven National Laboratory in New York; and the Macromolecular Diffraction Biotechnology Resource (MacCHESS) at Cornell University. This arrangement reflects the interdependence of complex research infrastructure across Federal agencies.

Technological advances made at NIH-supported synchrotron resourcesBcombined with enormously improved computing power and molecular biology technologies such as those involving recombinant DNABhave transformed x-ray crystallography from a laborious undertaking to a powerful research tool for molecular biology. In 1970, fewer than a dozen protein structures had been solved at atomic resolution; today that number surpasses 8,000, with solvable structuresBsuch as the ribosomeBbecoming increasingly large and complex. More than half of the almost 1,600 new structures solved in 1998 utilized synchrotron radiation.

One critical protein structure solved a decade ago led directly to discovery of the highly effective and widely used anti-AIDS drugs known as protease inhibitors. Scientists working in part at the NIH-supported MacCHESS synchrotron resource at Cornell University in New York determined the structure of the HIV protease at atomic resolution with candidate drugs that could fit snugly into the protease's active site and block its activities. Together with numerous similar studies at other synchrotrons and in home laboratories, this work led to development of the next generation of protease inhibitors, several of which are now in clinical use.

Genome scientists are shifting their focus from genetic structure to function, and structural biologists are increasingly pursuing the functional implications of molecular structure. Such studies received a significant boost in 1996 when the Advanced Photon Source (APS) near Chicago opened, a third-generation synchrotron that produces hard X rays that are two orders of magnitude brighter than those generated by older, second-generation synchrotrons.

Because synchrotron light is pulsed, it is ideal for time-resolved studies that produce brief "movies" of functioning molecules, capturing freeze frames of molecular motions that normally occur too rapidly to be seen. While older synchrotron sources also produce short pulses, they are not sufficiently bright to capture fast protein activities in real time. The new, third-generation synchrotrons like APS deliver extremely bright pulses that last for only 100 picoseconds, or trillionths of a second, each containing sufficient numbers of photons to record a "frame" of a movie. This allowed NIH-supported scientists to produce the world's first 3-D x-ray movie of a molecular reaction on a timescale of nanoseconds, or billionths of a second. The movie revealed how the molecule myoglobin changes its shape as it performs its primary functionBcapturing and releasing oxygen in muscle cells.

As the brilliant X rays continue to find new and unanticipated applications, demand for access to biomedical synchrotron resources will continue its exponential climb to unravel the molecular basis of disease and to facilitate novel drug development.

The DNA Chip

The flood of data emerging about DNA and genes demands new ways of sorting through the information to find the telling details that will illuminate how living beings function and that will advance medicine. Miniaturized Achip@ technologies, also called microarrays, are being used for many different applications. Some microarrays gauge how active different genes are in different kinds of cells; others let researchers track the molecular changes in tumor cells as cancer progresses; still others reveal DNA variations, including those that explain individual susceptibility to disease.

All microarrays share this characteristic: they permit researchers to examine many elements in parallel. NIH has supported the development of the major microarray technologies in use, including chips sold commercially, and has promoted communication among researchers in the field.

As more and more genes are discovered, the new-found abundance is propelling scientists out of the pattern of studying genes individually; instead, many are starting to monitor thousands of genes at a time. For such large-scale analyses, microarray technology can be rapid, efficient, and economical.

Expression arrays, which chart gene activity, have been among the most productive chips so far. To make an expression array, robots spot fragments from thousands of genes onto a single glass microscope slide. NIH researchers have used these arrays to chart which genes are turned on or off in cancer cellsBfor example, from the breast, prostate, and skinBcompared with normal cells. Expression arrays can also be used to examine how environmental exposure, such as an infection, can affect gene activityBthat is, whether a gene is mute in a cell or actively directing the production of proteins. For example, using expression arrays, NIH researchers are tracking how the DNA in blood cells reacts when infected with different strains of Ebola virus.

While expression arrays can reveal potentially important genes, for example in the development of cancer, a second kind of array, called the tissue microarray, can help establish the importance of each gene that emerges as a candidate for an important role. NIH researchers have developed a way of arranging some 1000 tiny cylindrical tissue biopsies in a small paraffin block. Thin slices cut from this block can be mixed with a probe that binds to a specific gene or gene product to allow researchers to visualize gene number, activity or subcellular localization of proteins in hundreds of different tissues simultaneously. Tissue arrays permit researchers to examine the molecular details (e.g. comparing gene expression patterns) of many different healthy tissue types or in different stages of disease.

In another major kind of chip, DNA molecules are manufactured right on a glass wafer using light-directed fabrication techniques that were originally developed to make semiconductor chips. NIH researchers have used these chips to identify single letter misspellings in the genes BRCA1, BRCA2, and ATM that can increase the risk of breast and ovarian cancer. Unlike some other disease genes where one or a few mutations account for nearly all cases of disease, for all three of these genes literally hundreds of misspellings have been discovered. The genes are very

large and finding the one unit that varies in a patient sample can be a complex and expensive task. But chips can highlight in a quick experiment whether a patient=s gene differs from the normal form, and if so, where the difference lies. The same kind of chips are being used to screen for mutations in *p53*, the gene most often mutated in human cancer cells, and in the AIDS-causing virus HIV, for example, to find what makes some forms of the virus drug resistant.

Chips are being used to find other DNA variations as well, the so-called SNPs (or single nucleotide polymorphisms) sprinkled throughout our chromosomes. These variations make each of us unique, shape our risk for diseases and account for why a drug may work for one person and not for another. Chips could even be used to work out the complete DNA code of some organisms, where a species is closely related to another species whose genetic code is already known. For instance, the complete DNA sequence in chimpanzees may be elucidated by comparison with that of the human as recently demonstrated at NHGRI by using the human BRCA1 chip to sequence the chimpanzee BRCA1 gene.

In all their various forms, microarray technologies can support the study of genetic complexity and are becoming increasingly common as a Agenome perspective,@ one that considers the entire DNA code of an organism, takes root in biomedicine.

Opening a Window on the Brain

The brain is a Aprivileged@ organ. Encased in the bony skull and cushioned by fluid, it is remarkably well-protected from the punishing toll of falls and knocks that can happen at all ages. This privileged status, however, comes at a priceBthe features that so effectively protect the brain from mishap also have prohibited scientists from observing directly what in the brain *can* go wrong through illness or severe injury. Until well into the 20th century, physicians and scientists have learned most of what was known about the brain from post-mortem studies and X-rays. Only recently have researchers possessed technologies capable of mapping the *structure* of the brain. Although neuroanatomic or structural information is vitally important, the brain can only be truly understood and appreciated in its dynamic, functioning state.

The challenge of understanding the *functioning* brain is compounded by its extraordinary complexity. Brain activity involves approximately 100 billion nerve cells, or neurons, all communicating amongst themselves and sending orders to and receiving messages from other parts of the body. In this communication process, electrical signals within neurons trigger chemical signals that diffuse across synapses, the tiny gaps that separate neurons. In total, there may be between 100 trillion and a quadrillion synapses in the brain. Over time, and as the result of multiple influences, enduring patterns of synaptic connections ultimately give rise to integrated neuronal Acircuits@ in the brain. Understanding what goes on at the brain=s neuronal level to turn large- and small-scale circuits on and off and, by pruning and strengthening synapses to reorganize circuits, is among the most urgent challenges confronting modern neuroscience. For scientists to understand behavior, mental activity, and consciousness at the most fundamental level, they must see and understand the brain at work.

For more than 100 years, scientists have known that an increase in blood flow in the brain is one marker of neuronal activity. Investigators subsequently linked blood circulatory changes to oxygen consumption by active brain cells. By the early 1950s, NIH-funded investigators had developed quantitative methods for measuring whole brain blood flow in humans. Examining the functional activity of neurons and circuits through such surrogate measures as circulation and blood oxygen alone, however, is much like analyzing the ocean floor through a glass-bottom boat. Thus, in recent decades, the pace has quickened in efforts to develop innovative technologies such as positron emission tomography (PET) scanning and functional magnetic resonance imaging (fMRI) that are capable of providing detailed maps of brain activity. Multiple NIH institutes contribute to and collaborate in these efforts.

Positron Emission Tomography (PET): The early 1970s brought the introduction of computed tomography, or CT scans, in which a series of x-rays generates two-dimensional images of soft tissue such as the brain. This innovation coincided with the development, by an NIH scientist, of a method, called a kinetic assay, that made it possible to quantify the metabolic activity of cells by attaching a radioactive tracer to glucose, which is the primary fuel for cellular activity. In the original animal studies, the amount of tracer-tagged glucose trapped inside a preserved cell could be captured on film, after the death of the animal, to depict quite accurately the level of cellular activity associated with a given behavior. These principles were subsequently extended to and validated in humans using tracer substances tagged with a short-lived radioisotope that, as it

decays, emits positrons that register their impact on sensitive scannersBthus the name, positron emission tomography (PET) scanning. When various radioisotopes are attached to natural substrates or to drugs that bind to particular neurotransmitter receptors, PET scanning affords scientists the capability to image the pharmacologic and biochemical aspects of neural function. By the 1980s, the merger of these technological strategies with innovative experimental designs for activating specific mental processes and dissecting human behaviors helped to establish PET as a centerpiece of the burgeoning field of cognitive neuroscience.

Functional Magnetic Resonance Imaging (fMRI): About the time that computed tomography and PET scanning were emerging, development of another imaging technology, known as magnetic resonance imaging began. MRI, in shorthand, works by measuring the absorption and emission of energy, but in this technology, the energy is electromagnetic, not radioactive. When a strong, uniform magnetic surrounds the body and a radio wave is passed through it, distortions in the magnetic field allow scanners to detect and image differences in the density and related features of various parts of the body. Building on earlier findings regarding the consumption of oxygen by neurons when they fire and the fact that red blood cells containing oxygen are intrinsically magnetic and thus able to perturb a larger uniform magnetic field, NIH-supported scientists and others in the late 1980s devised a method called Blood Oxygen Level Dependence, or BOLD, that lent itself to quanitfying brain activity. The combination of BOLD measurements with magnetic resonance technologies makes it possible to examine, non-invasively, the actual function of the brain by capturing an image at one point in time and then imaging changes associated with various types of mental activity over a short period of time.

These imaging technologies have accelerated the pace of discovery of brain research. Yet although the roots of functional brain imaging stretch back more than a century, the field is now entering its most exciting period of development. Accumulating experience is underscoring the advantages and limitations of each approachBthere are trade-offs between technologies. One, for example, is between the spatial precision, or clarity, of an image and the speed required for an imaging technology to capture the indescribably fast processing of information by the brain. Such realizations are adding impetus to the race to innovate. One direction of such innovation is to combine cutting edge technologies such as fMRI with tried-and-true techniques such as electroencephalography (EEG) and magnetoencephalography (MEG) that are easily able to detect electrical changes at the millisecond rate common to brain activity. Also in development are optical imaging technologies that promise both the spatial and temporal resolution of existing approaches at a fraction of the cost.

As science learns more about brain circuitry and learns more from cognitive neuroscience about how to activate and examine the function of particular brain circuits, differences between health and illness associated with the function of particular circuits certainly will become evident. Able to see precisely what goes wrong in what circuits and what synapses and with what chemical signals, scientists will be able to make increasingly safer medications that act with laser-like precision on affected circuits and neurotransmitter pathways and to Asee@ how a special kind of learning called psychotherapy works on the brain; to guide neurosurgical decisions; and to understand brain mechanisms involved in chronic, debilitating pain, among other uses. Without

impinging on the brain-s privileged status, functional imaging technologies are shedding light on the awesome secrets of how the brain works.